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Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients

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Abstract

Oxidative stress-mediated cell damage has been considered in the pathophysiology of schizophrenia. Abnormal findings have often been considered related to differences in ethnicity, life style, dietary patterns and medications, all of which influence indices of oxidative stress and oxidative cell damage. To minimize these confounds, schizophrenic patients were compared with age-matched control subjects with the same ethnic background and similar lifestyle, as well as with bipolar mood disorder (BMD) patients. Levels of antioxidant defense enzymes (i.e. superoxide dismutase, SOD; catalase, CAT; and glutathione peroxidase, GPx) were lower in schizophrenic patients than in controls, indicating conditions for increased oxidative stress. The contents of plasma thiobarbituric acid reactive substances (TBARS) were only marginally higher in schizophrenic patients, who had normal levels of arachidonic acid (AA), a major source of TBARS, indicating no significant oxidative membrane lipid peroxidation. Levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), however, were significantly lower in schizophrenic patients. When the same indices in BMD patients were compared with findings in matched controls, levels of only SOD and CAT were lower in the patients, whereas GPx was not. Again, as in schizophrenia, the contents of TBARS were marginally higher in BMD patients with no change in levels of AA. Levels of alpha-linolenic acid and EPA were significantly lower and levels of DHA were slightly lower in BMD patients. These data indicate that certain biochemical

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characteristics may be common to a spectrum of psychiatric disorders, and suggest supplementation of antioxidants and essential fatty acids might affect clinical outcome.

Keywords: Schizophrenia; Antioxidant enzymes; Lipid peroxides; Essential fatty acids; Bipolar mood disorder

1. Introduction

A contribution of oxidative stress-mediated cellular membrane pathology has been considered for a long time in the pathophysiology of schizophrenia (Mahadik and Mukherjee, 1996; Reddy and Yao, 1996; Smythies, 1997; Mahadik et al., 1999b; Yao et al., 2000; Kuloglu et al., 2002). Furthermore, treatment with some antipsychotics has also been considered to exacerbate the pathophysiology (Cadet and Lohr, 1987; Mahadik et al., 1999b; Parikh et al., 2002). Oxidative stress increases when the level of reactive oxygen species ($O_2^{\bullet -}$, $OH^{\bullet}$, $NO^{\bullet}$, $ONOO^{-}$, also known as free radicals) exceeds the cellular antioxidant defense capacity. The primary antioxidant defense is enzymatic, involving superoxide dismutase (SOD) catalase (CAT) and glutathione peroxidase (GPx). However, non-enzymatic antioxidant defense, which also plays a significant role in control of oxidative injury, constitutes dietary supplements such as antioxidant vitamins (A, C, E, Q), flavones, quinones, lycopenes, β-carotene and uric acid (Mahadik and Gowda, 1996; Mahadik et al., 1999a; Yao et al., 2000). Several studies of schizophrenia have reported abnormalities in one or more antioxidant enzymes and/or in the levels of cerebrospinal fluid (CSF) and plasma TBARS (Lohr et al., 1990; Mahadik and Mukherjee, 1996; Reddy and Yao, 1996). Several studies have also reported a wide range of abnormalities in membrane phospholipids and their fatty acids (Horrobin et al., 1994; Mahadik et al., 1996a; Peet et al., 1999; Horrobin, 1998) that may be a result of oxidative damage (Mahadik et al., 1999b; Yao et al., 2001). The correlations between the levels of these constituents and psychopathology have also been reported (Mukherjee et al., 1996; Mahadik et al., 1999b; Yao et al., 2000; Khan et al., 2002; Arvindakshan et al., 2003b). Furthermore, use of antioxidant vitamins alone (Lohr et al., 1988; Adler et al., 1993; Peet et al., 1993; Mahadik and Gowda, 1996; Mahadik and Scheffer, 1996; Yao et al., 2001) or EPUFA alone (Mahadik and Evans, 1997; Fenton et al., 2000; Peet and Horrobin, 2002) or a combination of these (Mahadik et al., 2001; Arvindakshan et al., 2003a) has been found to improve some of these psychopathologies. The disparate findings from these studies are considered due to differences in study subjects' ethnic origin, lifestyle, dietary patterns, socioeconomic status and use of medications, which have all been known to influence these indices (Mahadik and Gowda, 1996; Mahadik et al., 2001). This situation has made it difficult to assess the role of oxidative stress-mediated membrane pathology in schizophrenia and to establish its potential prevention/restoration by appropriate dietary supplementation.

The patients in these studies were treated primarily with older (conventional or typical) antipsychotics, which, due to their pro-oxidant activities (Jeding et al., 1995), are found to increase oxidative stress-mediated cellular injury in animals (Cadet and Lohr, 1987; Parikh et al., 2002). One group of investigators has reported changes in three key antioxidant enzymes (Mukherjee et al., 1996) and plasma lipid peroxides (Mahadik et al., 1998) in never-medicated patients within 5 days of first-episode of psychosis, indicating that antioxidant imbalance contributes to oxidative membrane injury. Recently, these investigators have found that the levels of lipid peroxides correlated with reduced levels of RBC membrane essential polyunsaturated fatty acids (EPUFAs) in the same patients (Khan et al., 2002). Furthermore, these investigators have found that the levels of lipid peroxides were significantly lower, and EPUFA levels were significantly higher, in patients with similar ethnic background treated with atypical, but not with typical antipsychotics.

This report and some other reports, in never-medicated (McCreadie et al., 1995) or in on–off-medicated (Yao et al., 2001) chronic schizophrenic patients, have provided additional evidence to support the contribution of oxidative stress-mediated membrane injury in the pathophysiology of schizophrenia and clinical outcome following its treatment with antipsychotics. One recent report by Akyol et al. (2002) has provided yet stronger evidence for oxidative stress-mediated lipid peroxidation based on reduced levels of plasma SOD, antioxidants (e.g. uric acid, bilirubins) and increased levels of xanthine oxidase, nitric oxide and lipid peroxides. However, the significance of plasma levels of antioxidant enzymes is unclear.

In our recent study, in schizophrenic patients from India, we found that the levels of RBC membrane n-6 EPUFAs were not lower and plasma lipid peroxides were not higher (Arvindakshan et al., 2003b) compared with levels in patients in the USA (Khan et al., 2002). These differences were reflected in the differences in severity of psychopathology (i.e. less severe symptomatology in Indian patients), which was evaluated using similar procedures by investigators mutually trained. Although several reports have indicated that patients in India have a milder form and better clinical outcome of schizophrenia, there has not been any explanation of this phenomenon (Jablensky, 1986; Jablensky and Häfner, 1986; Jablensky et al., 1991; Leff et al., 1992). However, possible contributions of type of diet and lifestyle have been implicated (Christensen and Christensen, 1988; Mahadik et al., 1999a; Arvindakshan, 2003).

We have now studied the key indices of both oxidative stress (i.e. antioxidant enzymes) and oxidative membrane damage (i.e. lipid peroxides and membrane fatty acids) in patients with well-matched normal subjects in India. All the study subjects had the same ethnic origin, lifestyle (hardly anybody smokes or drinks), and dietary patterns and do not change their domicile. Also, similar analyses were done in patients with bipolar mood disorder (BMD) matched with control subjects. BMD was considered an important comparison group since reduced levels of membrane EPUFA and improved clinical outcome by supplementation with n-3 EPUFA has been reported in BMD patients (Edwards and Peet, 1999; Maes et al., 1999). Moreover, an oxidative stress-mediated membrane phospholipid abnormality may be a plausible link to support the long-held concept of a continuum between schizophrenia and BMD (Maier et al., 1993; Crow, 1990, 1995).

2. Methods

2.1. Subjects

The patients evaluated were consecutive admissions to the outpatient treatment unit of Kripamayee Institute of Mental Health, Miraj, India, and from private hospitals in Pune, India. The study was performed in accordance with the Declaration of Helsinki (1964), which has been revised with a series of amendments including the last in Hong Kong (1989). The normal subjects consisted of healthy volunteers, who were evaluated using the Structured Clinical Interview for DSM Disorders, Non-patient Version (SCID-NP) and who were recruited from the general population and academic community via advertisement. Normals were matched for age and gender. Both patient groups and matched normal subjects had the same racial origin, very similar lifestyle and dietary patterns based on a dietary questionnaire and socioeconomic status (Arvindakshan, 2003). Urban vs. rural populations also had only minor variability in dietary pattern, due to strong traditional practices and the availability of the same food choices.

2.1.1. Inclusion criteria

Schizophrenic and BMD patients were screened for respective diagnostic criteria as derived from Structured Clinical Interview the Patient Version of the DSM-IV (SCID-P) (American Psychiatric Association, 1994). This study was designed to include subjects of both genders. However, due to socioeconomic factors that make it very difficult for female patients to seek/receive psychiatric care in India, only male subjects were available during the course of the study.

2.1.2. Exclusion criteria

For both normals and patients the exclusion criteria included: (1) WAIS-R full scale IQ $<$ 80;

Table 1
Demographic characteristics of schizophrenic and bipolar mood disorder (BMD) patients

	Schizophrenic patients $N=31$	BMD patients $N=10$
Mean age (years)	37.32±7.18[a]	40.8±8.29[b]
Weight (kg)	73.8±8.6	71.7±5.6
Age of onset (years)	24.04±8.14	30.5±6.69
Years of illness	12.84±8.10	10.75±6.25
BPRS	29.64±6.35	23.30±5.88
PANSS	54.71±12.67	NA
PANSS-negative symptom scores	16.90±5.61	NA
PANSS-positive symptom scores	10.65±4.22	NA

All values are mean±S.D.
[a] Mean±S.D. age (years) and weight (kg) of the matched controls for schizophrenic patients: 39.72±8.87 and 65.8±3.9, respectively.
[b] Mean±S.D. age (years) and weight (kg) of the matched controls for BMD patients: 40.9±8.84 and 67.7±4.9, respectively.

(2) high levels of dietary supplements; (3) severe undernourishment or malnurishment; (4) seizure disorder; (5) head injury with loss of consciousness; (6) alcohol and substance abuse or dependence, which was extremely rare in these patients; (7) type II diabetes; (8) lipid disorders; (9) cardiovascular disease; and (10) hypertension or family history of the same. These factors and obesity, which is also rarely seen in these patients, are known to affect the oxidative stress and membrane EPUFA status. Normals were also excluded for use of any medication for other medical problems.

2.1.3. Demographic characteristics of study subjects

Demographic characteristics of study subjects are included in Table 1. The mean age of control subjects ($N=27$) matched to schizophrenics ($N=31$) was 39.72±8.87 and $N=10$ matched to BMD patients ($N=10$) was 40.9±8.29, but did not significantly differ. Similarly, body weight of both the patient groups was slightly higher but did not significantly differ. Greater body weight may be due to atypical antipsychotic medication for the majority of patients. The body weight (Table 1) and the biochemical blood profile (Table 2) indicated that all the patients were medically healthy. The patients were adequately treated with medications (i.e. average of 3.5 medications with many on up to two antipsychotics, primarily atypicals such as risperidone, clozapine or olanzapine and two antidepressants such as depakote, celexa and loranzapine) as per the choice of their physicians. Most of the patients were on stable medication for at least a month. Schizophrenic patients showed average residual BPRS (29.64±6.35) and PANSS

Table 2
Biochemical blood data of controls, BMD and schizophrenics

Parameter	Normal range	Total controls ($n=31$)	BMD patients ($n=10$)	Schizophrenics ($n=31$)
Cholesterol	<200 mg	187.87±43.66	184.90±55.31	189.84±40.16
HDL cholesterol	>35 mg	41.0±8.83	40.0±11.89	42.52±10.38
LDL cholesterol	<130 mg	114.45±35.10	109.90±40.34	123.52±32.70
VLDL cholesterol	5–51 mg	32.32±22.61	35.0±21.08	23.81±13.34
Triglycerides	<200 mg	162.13±112.63	174.50±105.20	119.19±66.46
Lp(a)	<30 mg/dl	31.66±29.29	32.73±28.14	46.09±34.33
Apo A1	104–225 mg/dl	123.93±53.01	137.17±56.89	109.02±33.59
Apo B	60–133 mg/dl	115.74±44.79	124.20±45.96	99.59±38.82

All values are mean±S.D.

total (54.71 ± 12.67). These scores were much lower than we had found in never-medicated patients (42 ± 9.3; 80 ± 11.1, respectively) (Arvindakshan et al., 2003b). Most of the BMD patients were asymptomatic at the time of enrollment. Three had marginal HAMD scores. However, they had significant BPRS scores, indicating positive psychotic symptomatology.

2.1.4. Clinical assessments

Schizophrenic patients were rated for psychopathology using both the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962). The BPRS is more suitable for correlation studies with biochemical measures, since it provides focused subjective rating. BMD patients were rated on the BPRS for psychotic symptoms and on the Hamilton Depression (HAMD) Rating Scale using the Structured Interview Guide for Mood Disorders (Williams, 1988). The assessments were carried out within 1 week of enrollment by trained psychologists, who were tested at intervals for inter-rater reliability with one of the co-authors (Dr Madhav Ghate). Dr Ghate has established inter-rater reliability (> 0.89) with an investigator in the USA (Dr Denise Evans, Veterans Affairs Medical Center, Augusta, GA) by assessing the patients in the USA as well as in India. Drs Denise Evans and Matcheri Keshavan (Department of Psychiatry, University of Pittsburgh, PA, USA) have been consultants on this project for the last 5 years. They have visited the research center in India, discussed the issues related to clinical assessments, and have ongoing input. Assessments were based on the information from the patient, a reliable informant accompanying the patient and hospital records.

2.1.5. Approval by Institutional Review Board (IRB)

The research protocols and consent forms were approved by the IRB of both the Kripamayee Institute for Mental Health, Miraj, and the Poona Hospital, Pune, India. These protocols were also approved by the IRB, Medical College of Georgia, Augusta, GA, and by the Division of Human Subject Protections, Office of Protection from Research Risks (OPRR), NIH, USA. All the subjects consented to the study and signed consent forms.

2.2. Procedures

2.2.1. Collection and processing of blood samples

Fasting venous blood was collected immediately after enrollment in tubes containing EDTA. RBCs and plasma were separated by centrifugation, coded and stored at −70 °C until used for fatty acid analysis, TBARS and antioxidant enzyme assays, respectively. Laboratory investigators were blind to the identity of the subject (patient vs. normal), which was indicated by a code number maintained by the clinical staff until all biochemical analyses were completed.

2.2.2. Antioxidant enzyme assays

Hemolysates were prepared by mixing 0.1 ml of washed RBC with 0.4 ml of cold distilled water and vortexed for a few seconds. RBC plasma membranes were removed by centrifugation at 10 000 × g rev./min for 30 min at 4 °C, and clear hemolysates were used for analyses of antioxidant enzyme, e.g. SOD, GPx and CAT.

2.2.3. Assay for catalase

The CAT assay was carried out essentially by Aebi's spectrophotometric method (Aebi, 1984). Briefly, first 1 ml quartz cuvette contained in 1 ml phosphate buffer (pH = 7.0, 50 mM) and 10 μl of hemolysate (suitably diluted, if required, with phosphate buffer) were added and the spectrophotometer was adjusted to auto zero. To a second similar cuvette, 3 mM hydrogen peroxide (freshly diluted) was added, the decrease in absorbance at 240 nm was observed for 1 min and the activity was calculated as $\mu M\ min^{-1}\ l^{-1}$.

2.2.4. Assays for SOD and GPx

The activities of SOD and GPx were determined using BIOXYTECH kits from Oxis International, Inc (Portland, CA, USA). The protocols were followed as per the direction in the manufacturer's manual, and activities of SOD and GPx were expressed as units per milliliter of hemolysate and

milli-units per milliliter of hemolysate, respectively.

2.2.5. Analysis of plasma TBARS

Ohkawa's method (Ohkawa et al., 1979) was used to measure plasma TBARS. Briefly, 3 ml of reaction mixture contained samples, sodium dodecyl sulfate (0.41%), acetic acid (7.5%; pH 3.5) and thiobarbituric acid (0.3%, freshly prepared) in glass tubes. The tubes were covered with aluminum foil and heated at 95 °C for 60 min. After adding 1 ml of distilled water, TBARS were extracted in 3 ml of butanol. The absorbance of the butanol layer was measured at 532 nm. The standard, tetra methoxy propane was used as the reference peroxide and values were expressed as nM/ml. Although this procedure estimates peroxides from both lipids and non-lipid source, we earlier found that these values were consistent with values estimated by high performance liquid chromatography (Mahadik et al., 1998).

2.2.6. RBC membrane fatty acid analysis

RBC samples of the controls, schizophrenic patients and BMD patients were analyzed by gas chromatography as we have recently described (Arvindakshan et al., 2003a,b). Briefly, 1 ml of RBC was taken in a 15-ml screw cap vial and 4.0 ml of 0.6 N methanolic HCl containing 4 μl of 0.5% BHT were added. The vials were sealed and incubated at 80 °C for 2 h. After incubation, methylated fatty acids were extracted twice (2 and 1 ml each time) with hexane, and layers were separated by centrifugation in a swinging rotor at $3000 \times g$ rev./min for 15 min at room temperature. The hexane layers were carefully removed and collected in a separate vial. The hexane extract was completely dried by passing argon and stored at −40 °C until analyzed.

The methylated fatty acids were resuspended in 50 μl of chloroform and 1 μl was analyzed by gas chromatography performed on a Shimadzu gas chromatograph, model GC 17 A Ver 3 using a capillary column of 30 m×0.32 mm×0.20 μm dimensions (Supelco, USA). A flame ionization detector (FID) was used with a column oven temperature at 175 °C for 15 min, programmed at 10 °C rise/min up to 220 °C and finally held at 220 °C for 10 min. The temperature of the injector was 240 °C and the detector temperature was set at 275 °C. The column was calibrated by injecting the standard fatty acid mixture in approximately equal proportions. The data were recorded and the peaks were identified as per the retention time of the standard fatty acids run under the identical conditions. The data are presented as percent of the total fatty acids: saturated fatty acids (SFA): myristate C14:0, palmitate C16:0, stearate C18:0); monosaturated fatty acids (oleate C18:1) and polyunsaturated fatty acids: n-6 fatty acids (linoleate C18:2, gamma linoleate C18:3, arachidonate C20:4) and n-3 fatty acids (alpha linoleate C 18:3, eicasopetanoate C20:5, docosahexaenoate C22:6).

2.3. Statistical analysis

All the procedures were standardized to yield reliable and reproducible (coefficient of variance = 5±2) values. Replicate measures were done for each subject, and the average of the nearest two values was used for statistical analysis. Kruskal–Wallis analysis of variance (ANOVA) was used for between-group comparisons. Correlation between variables was studied using Pearson's correlation analysis. When the data were non-normally distributed, non-parametric statistics were employed to analyze the data. This study was designed to test the hypothesis that altered endogenous oxidative stress (i.e. antioxidant enzymes) will be associated with increased membrane pathology (i.e. increased lipid peroxides and reduced RBC membrane fatty acids) in patients with schizophrenia as well as bipolar disorder.

3. Results

3.1. Indices of oxidative stress and oxidative membrane damage in schizophrenia

3.1.1. Levels of antioxidant enzymes

The RBC levels of the three key antioxidant enzymes, the key indices of oxidative stress, were significantly lower in schizophrenic patients compared with control subjects: SOD, 1961±959 vs. 2892±1630, $F = 3.746$, d.f. = 30,26, $P = 0.009$; CAT, 2080±301 vs. 2330±306, $F = 8.984$, d.f. =

Table 3
Antioxidant enzymes in control subjects and schizophrenic patients

	Control subjects ($N=27$)	Schizophrenic patients ($N=31$)
Superoxide dismutase (U/ml)	2892 ± 1630	1961 ± 959 $P=0.009$[a] $F=3.746$ d.f. = 30,26
Catalase (μM min^{-1} l^{-1})	2330 ± 306	2080 ± 301 $P=0.003$[a] $F=8.984$ d.f. = 30,25
Glutathione peroxidase (mU/ml)	258 ± 146	163 ± 66 $P=0.013$[a] $F=13.039$ d.f. = 30,25

All values are mean ± S.D.
[a] Statistically significant differences ($P=<0.05$) between schizophrenics and normal subjects were determined by ANOVA.

30,25, $P=0.003$; GPx, 163 ± 66 vs. 258 ± 146, $F=13.039$, d.f. = 30,25, $P=0.013$ (Table 3). However, none of the enzymes were correlated with age or symptoms.

3.1.2. Levels of plasma TBARS and RBC membrane EPUFA

Plasma TBARS showed a trend towards increase but did not significantly differ from normal values (Table 4). Similarly, none of the n-6 fatty acids, particularly AA, a major source of plasma TBARS, were reduced in patients. However, the levels of all three major n-3 fatty acids, viz. ALA, EPA and DHA, were significantly lower in patients ($F=3.277$, 1.605, 3.626; d.f. = 30,26 for all, and $P=0.016$, 0.026 and 0.034, respectively) compared to matched controls.

3.2. Indices of oxidative stress and oxidative membrane damage in BMD

3.2.1. RBC antioxidant enzymes

Table 5 shows the levels of antioxidant enzymes in BMD patients and matched controls. The levels of SOD were slightly lower ($F=10.916$, d.f. = 8,9, $P=0.054$). The levels of CAT were significantly lower ($F=11.089$, d.f. = 9,9, $P=0.025$) and those of GPx were slightly but not significantly higher ($F=3.786$, $P=0.624$).

3.2.2. Plasma TBARS and RBC membrane EPUFAs

The levels of plasma TBARS were marginally higher in BMD patients compared to matched controls (Table 6). Again, the levels of n-6 fatty acids including AA were not altered in BMD patients. However, the levels of n-3 fatty acids, ALA and EPA were significantly lower ($F=3.79$ and 8.004; d.f. = 1,18 and 9,9, $P=0.041$ and 0.029, respectively) and the level of DHA was slightly lower in patients compared to matched controls.

4. Discussion

The data from the present study have led to the following key findings: (1) In schizophrenic patients, the levels of all three antioxidant defense enzymes (SOD, CAT, and GPx) were significantly lower, indicating a situation for increased oxidative stress. There was no increase in lipid peroxides, TBARS, which was supported by no change in n-6 EPUFAs, a primary source of TBARS. However, the levels of n-3 EPUFAs were significantly lower. These changes were not related to age or years of illness. No correlation was found between levels of any antioxidant enzyme or TBARS or any fatty acid and clinical PANSS-PSS or PANSS-NSS or BPRS score. (2) In BMD patients, the levels of two antioxidant defense enzymes (SOD and CAT)

Table 4
RBC membrane fatty acids and plasma TBARS in control subjects and schizophrenic patients

	Control subjects ($N=27$)	Schizophrenic patients ($N=31$)
SFA	38.45±3.47	39.01±9.03
T n-9 FA	11.28±1.46	11.47±3.89
T n-6 FA	31.23±3.35	28.14±7.00
T n-3 FA	3.02±2.37	1.86±1.23 $P=0.007$[a] $F=4.766$; d.f.=30,30
AA	16.19±2.06	16.08±3.97
ALA	0.98±2.23	0.36±0.26 $P=0.016$[a] $F=3.277$; d.f.=30,26
EPA	0.72±0.51	0.55±0.81 $P=0.026$[a] $F=1.605$; d.f.=30,26
DHA	1.33±0.92	0.95±0.90 $P=0.034$[a] $F=3.626$; d.f.=30,26
TBARS (nM/ml)	3.23±1.07	4.06±1.79

All values are mean±S.D.

T SFA-Total saturated fatty acids=C14:0, C16:0, C18:0, C20:0, C22:0, C24:0; C=carbons.

T n-9 FA-Total n-9 fatty acids=C18:1n-9, C20:1n-9, C20:3n-9, C22:1n-9, C24:1n-9.

Tn-6 FA-Total n-6 fatty acids=C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6, C22:5n-6.

Tn-3 FA-Total n-3 fatty acids=C18:3n-3, C18:4n-3, C20:3n-3, C20:5n-3, C22:5n-3, C22:6n-3.

AA, arachidonic acid; ALA, Alpha-linolenic acid; EPA, Eicosahexaenoic acid; DHA, docosahexaenoic acid; TBARS, Thio-Barbituric Acid Reactive Substances.

Values are expressed as mean±S.D.% of total fatty acids.

[a] Statistically significant differences ($P=<0.05$) between schizophrenics and normal subjects were determined by ANOVA.

were lower, indicating an increased oxidative stress, but again there was no change in levels of n-6 EPUFAs and levels of n-3 EPUFAs were significantly lower. (3) These data indicate that, in both groups of patients, increased oxidative stress is not contributing to the increased membrane lipid peroxidation and reduced levels of n-6 EPUFAs. The decreased n-3 EPUFAs may be related to some other factors such as defective conversion of precursors to DHA (Mahadik et al., 1996b) or increased PLA2 (Horrobin, 1998; Mahadik et al., 1999b) or dietary inadequacy (Mahadik et al., 1999a), since dietary supplementation was found to normalize the levels of EPA and DHA (Arvindakshan et al., 2003a). It is important to point out that increased oxidative stress might still cause other forms of oxidative cellular damage, e.g. DNA breakdown and inactivation of proteins/enzymes or mitochondria, which have been also suggested to contribute to the pathophysiology of schizophrenia. These findings in schizophrenic and BMD patients further suggest that these disorders probably share a biochemical pathology.

4.1. Studies in schizophrenic patients

This is the first study to measure simultaneously key indices of oxidative stress (i.e. antioxidant enzymes and lipid peroxides) and membrane oxidative damage (i.e. reduction of membrane phospholipid polyunsaturated fatty acids) in the same subjects. In chronic medicated (primarily with typical antipsychotics such as haloperidol, prolixin, and chlorpromazine) schizophrenics, we reported that the levels of SOD were higher, CAT lower and GPx unchanged (Reddy et al., 1991). However, later in never-medicated first-episode psychotic patients, we reported that levels of SOD were lower but no change in the levels of CAT and GPx (Mukherjee et al., 1996). Data from these two studies suggested that treatment with typical antipsychotics might alter the balance between these enzymes: increase the SOD and CAT but not GPx. However, Yao et al. (1998) have reported higher SOD in patients on or off haloperidol treatment, and Akyol et al. (2002) have reported reduced plasma SOD in patients with low or very high dose neuroleptics. It was concluded that haloperidol or other antipsychotics may not have a direct effect on antioxidant enzymes and other factors including severity of symptomatology may influence activities of, particularly, SOD and GPx. These authors also found that smoking status did not influence the levels of these enzymes. The effects of medications are always difficult to investigate in patients, since these cannot be studied prospectively for an extended period of time. Recently, we found that levels of these enzymes

Table 5
Antioxidant enzymes in control subjects and bipolar mood disorder (BMD) patients

	Control subjects ($N=10$)	BMD patients ($n=10$)
Superoxide dismutase (U/ml)	3044 ± 1411	1481 ± 1870 $P=0.054$[a] $F=10.916$ d.f. = 9,8
Catalase (μM min^{-1} l^{-1})	2273 ± 274	2013 ± 195 $P=0.025$[a] $F=11.089$ d.f. = 9,9
Glutathione peroxidase (mU/ml)	209 ± 122	241 ± 98 NS

All values are mean ± S.D.
[a] Statistically significant differences ($P = <0.05$) between schizophrenics and normal subjects were determined by ANOVA.

were lower at the onset of psychosis and increased by 6 months of treatment with either risperidone or olanzapine (Evans et al., 2003). The schizophrenic patients in the present study were receiving an average of 3.5 medications that included predominantly atypical antipsychotics, e.g. risperidone, clozapine and olanzapine; however, none of the patients smoked significantly. Antipsychotics, particularly typicals, are considered to have prooxidant effects (Jeding et al., 1995), probably by affecting the expression of antioxidant enzymes (Roy et al., 1984; Cadet and Perumal, 1990; Parikh et al., 2002) in addition to converting into free radicals, as in the case of haloperidol (Subramanyam et al., 1991). However, atypicals such as clozapine, risperidone or olanzapine have not been found to reduce the expression of these antioxidant enzymes in rat brain (Parikh et al., 2002). These reports and findings of our present study suggest that the levels of antioxidant enzymes may be generally lower at the very early stages of psychotic illness, and antipsychotic treatments may alter further, depending on the type of medication, severity of psychopathology or environmental factors such as diet and socioeconomic status. Since patients in this study are racially homogeneous, are similar in their lifestyle and dietary pattern, and were on stable medication at the time of enrollment, lower levels of antioxidant enzymes most likely are related to the severity of illness ('state' markers).

Plasma lipid peroxides, one of the key indices of membrane pathology, were not increased in this study, though several reports have shown an increase in drug-naïve first-episode patients compared to chronic schizophrenic patients (Lohr et al., 1990; Mahadik et al., 1998; Khan et al., 2002; Arvindakshan et al., 2003b; reviews Mahadik and Mukherjee, 1996; Reddy and Yao, 1996; Mahadik et al., 1999b). It is likely that plasma lipid peroxides reflect the membrane n-6 EPUFAs, particularly AA, a major source and highly susceptible for peroxidation (Ramchand et al., 1996). Since AA levels were not reduced, that may explain the lack of an increase in lipid peroxides. We have earlier reported similar data in a separate cohort (Arvindakshan et al., 2003a, 2003b). These data suggest that reduction of AA with an increase in peroxides may be a result of oxidative stress due to clinical state, lifestyle and dietary antioxidants, which protect AA in these study patients, in addition to decreased antioxidant enzymes.

This study found that the levels of n-3 fatty acids, particularly DHA, were significantly reduced, which is consistent with several published studies (reviewed by Horrobin et al., 1994; Mahadik and Evans, 1997; Horrobin, 1998; Khan et al., 2002), including in a separate similar cohort (Arvindakshan et al., 2003b). It has been suggested that reduced AA is associated primarily with lipid peroxidation (Mahadik et al., 1999a), since it is adequately available in diets around the world.

Table 6
RBC membrane fatty acids and plasma TBARS in control subjects and bipolar mood disorder (BMD) patients

	Control subjects ($N=10$)	BMD patients ($N=10$)
T SFA	38.71 ± 3.42	36.27 ± 3.13
T n9 FA	11.70 ± 1.07	11.03 ± 1.87
T n-6 FA	30.38 ± 3.06	30.02 ± 3.58
T n-3 FA	2.26 ± 1.14	1.26 ± 0.42 $P=0.018$[a] $F=4.35$; d.f. = 10,10
AA	15.90 ± 1.31	16.13 ± 1.94
ALA	0.49 ± 0.37	0.21 ± 0.18 $P=0.041$[a] $F=3.79$; d.f. = 1,18
EPA	0.53 ± 0.38	0.19 ± 0.26 $P=0.029$[a] $F=8.004$; d.f. = 9,9
DHA	1.23 ± 0.93	0.87 ± 0.48
TBARS (nM/ml)	3.05 ± 0.86	3.93 ± 2.36

All values are mean ± S.D.

T SFA-Total saturated fatty acids = C14:0, C16:0, C18:0, C20:0, C22:0, C24:0; C = carbons.

T n9 FA-Total n9 fatty acids = C18:1n9, C20:1n9, C20:3n9, C22:1n9, C24:1n9.

Tn-6 FA-Total n-6 fatty acids = C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6, C22:5n-6.

Tn-3 FA-Total n-3 fatty acids = C18:3n-3, C18:4n-3, C20:3n-3, C20:5n-3, C22:5n-3, C22:6n-3.

AA, arachidonic acid; ALA, Alpha-linolenic acid; EPA, Eicosahexaenoic acid; DHA, docosahexaenoic acid; TBARS, Thio-Barbituric Acid Reactive Substances.

Values are expressed as mean ± S.D.% of total fatty acids.

[a] Statistically significant differences between BMD patients and control subjects were determined by ANOVA.

Recently, we found that EPA and DHA levels were lower but there was no change in AA at the onset of psychosis, and 6 months of atypical antipsychotic treatment increased the levels of EPA and DHA (Evans et al., 2003). Reduced DHA levels might be related to several factors indicated earlier such as lower availability in diets, increased metabolism or some defect in utilization of its precursors in schizophrenia. Supplementation with n-3 fatty acids (EPA and DHA) in a separate similar cohort was found effective in ameliorating psychopathology (Arvindakshan et al., 2003a). Reduced levels of n-3 EPUFA is an important finding, since these are critical for brain and behavioral development and membrane function throughout life (Simopoulos, 1991; Wainwright, 1992).

It is possible that, in the Indian population, with a predominantly rural culture and a low caloric diet rich in fruits and vegetables, oxidative stress may be low, thereby keeping low levels of antioxidant enzymes, which are known to increase under increased oxidative stress. Therefore, the lower levels of all these antioxidant enzymes and of n-3 EPUFAs in this unique study population, strongly suggest that both of these biochemical processes may be associated with different mechanisms in the pathophysiology of schizophrenia.

None of these biochemical indices of oxidative stress and oxidative cell injury showed correlation with the various symptom scores. It has been indicated that, in schizophrenia, positive symptoms and negative symptoms may be related to different biochemical processes (Crow, 1985; Andreasen and Olsen, 1982). Glen et al. (1994) reported that reduced levels of both AA and DHA were present in patients with predominant negative symptoms and not in patients with predominant positive symptoms. Recently, based on extensive work by many investigators, it has been suggested that psychopathology with predominant negative symptoms, i.e. core negative symptoms, may be defined as a deficit syndrome (Carpenter et al., 1988). The patients in this study had predominantly negative symptoms (PANSS-negative symptom scores, 16.90 ± 5.61) and lower positive symptom scores (PANSS-positive symptom scores, 10.65 ± 4.22). Patients with the deficit syndrome have been reported to have a sustained psychopathology (Kirkpatrick et al., 1993), which may be related to structural brain abnormalities possibly resulting from lower n-3 fatty acids in early stages of illness. The psychopathology associated with structural brain abnormalities may not correlate with the present levels of peripheral indices. However, these may indicate the risk for severe psychopathology, if oxidative stress is further increased.

4.2. Studies in BMD patients

In BMD, antioxidant enzymes, plasma lipid peroxides and membrane fatty acids were also

analyzed for the first time. Though the number of subjects is small, the levels of SOD and CAT were significantly lower and the level of GPx was unchanged. These data differ from a few published reports, where SOD was reported higher (Abdalla et al., 1986) and GPx was reported lower (Stoklasova et al., 1990). Similar to findings in schizophrenia, plasma lipid peroxides were again marginally higher with no change in AA and levels of n-3 fatty acids were lower similar to earlier reports (Edwards and Peet, 1999; Maes et al., 1999). Only three out of 10 patients had significant HAMD scores but the group had significant positive psychotic symptomatology at the time of enrollment. Replication of data with a large number of patients will establish the conditions for the proper use of supplementation for depressive disorders (Stoll et al., 1999; Nemets et al., 2002).

4.3. Possible biochemical link between schizophrenia and BMD

Data in schizophrenic and BMD patients may shed light on the molecular mechanism for the proposed common membrane phospholipid pathology in a broad spectrum of psychiatric disorders (Peet et al., 1999). They may also provide evidence to support the long-held concept of a continuum between mood and schizophrenic disorders and between normal and pathological behavior (summarized by Angst, 2002). Based on Kahlbaum's (1963) classification, Kraepelin (1899) proposed a dichotomy between 'manic-depressive insanity' and 'dementia praecox'. However, over the years to the present, the debate goes on (Maier et al., 1993; Crow, 1990, 1995; Taylor, 1992). Family studies (Gershon et al., 1988; Maier et al., 1993) as well as genetic linkage studies have not been able to disprove the hypothesis of a continuum (Crow, 1990). Crow (1995) has postulated that 'there are no disease entities but the psychosis can be regarded as 'boundary' conditions of continuous variation that is present in the general population'. In these regards, data in this unique study population may be very important, since except for psychiatric classification, the rest of the factors were identical in both patient groups and their matched controls.

Finally, it is important to indicate that oxidative stress-mediated membrane pathology can be effectively corrected by dietary supplementation and clinical outcome significantly improved (Mahadik et al., 2001). This is particularly important, since Khan et al. (2001) reported that conventional treatments for the last 50 years have slightly improved the most troubling symptoms but have not significantly improved the quality of life of these patients in terms of rehabilitation in the community and employment.

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