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RESEARCH ARTICLE

Oxidative stress in Schizophrenia patients with and without diabetes mellitus.

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ABSTRACT

Introduction: In recent times schizophrenia has shown positive association with diabetes mellitus. Previous studies have suggested possible role of oxidative stress in pathophysiology of neuropsychiatric disorders. In the current work, we have measured oxidative damage to biomolecules in schizophrenia patients with and without diabetes mellitus to know the strength of association of schizophrenia with diabetes mellitus.

Materials and methods: Serum samples from 39 patients having schizophrenia without diabetes mellitus (group I) and 21 patients having schizophrenia with diabetes mellitus (group II) and 50 healthy controls were collected to analyze lipid peroxidation marker malondialdehyde (MDA) and major antioxidant total thiol levels using colorimetric methods. Fasting blood glucose (FBG), serum urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin and direct bilirubin were determined by automated analyzer Hitachi 912.

Results: There was significant increase in FBG ($P<0.001$) and MDA (<0.001), and decrease in total thiols ($p<0.001$) in group I compared to healthy controls. There was significant increase in (FBG) ($p<0.001$) and MDA ($p<0.001$), decrease in total thiols ($p<0.001$) in group II compared to group I and healthy controls. However, there was no significant difference in serum urea, creatinine, AST, ALT, total bilirubin and direct bilirubin between patients and healthy controls. Serum MDA levels correlated negatively with total thiol levels ($r = -0.275$, $p<0.01$) and positively with FBG ($r = 0.823$, $p<0.01$) in group II patients.

Conclusion: Our study has shown presence of oxidative stress in schizophrenia which is further enhanced in schizophrenia associated with diabetes mellitus.

Keywords: Schizophrenia, total thiols, malondialdehyde, diabetes mellitus, oxidative stress.

1. INTRODUCTION

Schizophrenia is a common psychiatric disorder, marked by gross distortion from reality: disturbances in thinking, feeling and behavior. (1) It most commonly manifests in young adulthood and presents with auditory hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking with significant social or occupational dysfunction. (2) Studies suggest that genetics, environmental, neurobiology, psychological and social processes are important contributory factors in development of the disease. (3) Current psychiatric research had indicated increased dopamine activity in the mesolimbic pathway of the brain in etiology of schizophrenia and which becomes mainstay of treatment in antipsychotic medication; works by suppressing dopamine activity .(4) The prevalence of schizophrenia

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ranges from 1% to 2% in the United States, 2.3% in India and point prevalence of 3-4% worldwide (5, 6, 7).

Data from several reports indicate that free radicals are involved in pathogenesis of number of human pathologies including neuropsychiatric disorders such as schizophrenia. (8) The potential toxicity of free radicals is counter acted by a large number of cytoprotective enzymes and antioxidants that limit the damage. (9) Malondialdehyde (MDA) is a secondary lipid peroxidation end product and is specific marker of lipid peroxidation of membrane. Increased MDA implicated in the pathogenesis of schizophrenia and other neuropsychiatric disorders. (10, 11) Total thiols are the major antioxidants in the body and they consists of free sulfhydryl (-SH) groups in plasma and cells, and –SH groups bound to proteins. Protein thiols in the plasma include the protein sulfhydryl groups (-SH) and protein mixed disulphides with homocysteine, cysteinylglycine, cysteine and glutathione. (12) As a main cellular nonprotein antioxidant and redox regulator, glutathione (GSH) plays a major role in protecting nervous tissue against reactive oxygen species and in modulating redox-sensitive sites, including N-methyl D-aspartate (NMDA) receptors (NMDA-R) considering the NMDA receptor hypo function is hypothesis for schizophrenia. (13)

Number of studies in patients with schizophrenia has found a positive association between schizophrenia diabetes mellitus. This has been thought to result from numerous factors associated with schizophrenia i.e the disease itself and its genetic factors, antipsychotic medication, lifestyle changes, and possibly weight gain may play a role. Some of studies proposed increased prevalence of diabetes mellitus in schizophrenia which is induced by hyperglycemia causing depletion of antioxidants, polyunsaturated fatty acids and generation of lipid peroxide intermediates leading to neuronal damage. (14) After extensive literatures search we could not found any studies showing difference in the levels of oxidative stress markers in patients with and without diabetes mellitus. In the present study, we have tried to determine the levels of oxidative stress in schizophrenia with and without diabetes mellitus, by analyzing MDA and total thiols, and to know if the determined parameter relates to each other.

Materials and Methods

Subjects and Samples

The study was carried out on 60 patients diagnosed with schizophrenia and 50 healthy controls. The patients were undergoing treatment at Dr A V Baliga Memorial hospital, Udupi. The mean age of patients with schizophrenia was 58±12 years and that of healthy controls was 52±12 years. The patients were grouped into schizophrenia without diabetes (group I, n=39) and schizophrenia with diabetes mellitus (group II, n=21). Average duration of disease is about 18±4 months and all the patients were on treatment with atypical antipsychotics. There were 42 males and 18 females in the patient group. The healthy controls were not on any kind of prescribed medication or dietary restrictions. Informed consent was taken from all subjects involved in the study and was approved by institutional review board.

Blood samples in fasting state, 2 ml was drawn into vacutainers containing anticoagulant and sodium fluoride, and 5 ml was drawn into plain vacutainers from the ante-cubital veins of healthy controls and schizophrenia patients. The blood samples drawn in vacutainers containing anticoagulant was analyzed within 30 minutes for glucose levels. The blood samples drawn in plain vacutainers was allowed to clot for 30 min and centrifuged at 2000g for 15 min for clear separation of serum.

Biochemical determinations

Special chemicals like 5' 5' dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), and standard MDA were obtained from sigma chemicals, St Louis, MO, USA. All other reagents were of chemical grade.

Total thiol assay

Reaction mixture contained 900 µL 2 mM Na_2 EDTA in 0.2 M Na_2HPO_4, 20 µL 10 mM DTNB in 0.2 M Na_2HPO_4 and 100 µL of serum. Reaction mixture was incubated at room temperature for 5 min; absorbance read at 412nm. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate serum total thiols using the molar extinction coefficient 1600 M^{-1} cm^{-1} and values expressed as µM. The calibration curve was produced using GSH dissolved in Phosphate buffered saline (PBS). (15)

MDA assay

Reaction mixture contained 1 mL 0.67% thiobarbituric acid (TBA), 500 µL 20% Tri carboxylic acid (TCA) and 100 µL serum. Incubated at 100^{o}C for 20 minutes; centrifuge at 12,000 rpm for 5 minutes. Absorbance of supernatant read at 532 nm. MDA was determined by using molar extinction coefficient 1.56 x 10^5 M^{-1} cm^{-1} and values expressed as µM. (16)

Other biochemical parameters

Fasting blood glucose (FBG), serum levels of urea and creatinine, aspartate transaminase and alanine transaminase, total bilirubin and direct bilirubin, levels were determined by automated analyzer Hitachi 912.

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). A $p<0.05$ was considered statistically significant. Statistical analysis was performed using the

statistical package for social sciences (SPSS-16, Chicago, USA). One way ANNOVA used to compare the mean between the groups. Pearson correlation was applied to correlate between the parameters.

Results

As depicted in table 1, there was a significant increase in serum MDA levels ($p<0.001$) and FBG ($p<0.001$) and decrease in total thiols ($p<0.001$) in group II patients compared to group I patients and healthy controls. We found significant elevation of MDA levels ($p<0.001$) and decrease in total thiols ($p<0.001$) in group I patients as compared to healthy controls. As depicted in table 1, other routine biochemical parameters did not show any significant difference between healthy controls and schizophrenic patients. On applying Pearson's correlation serum MDA levels correlated negatively with serum total thiols ($r = -0.275$, $p<0.01$) and positively with FBG (($r = 0.823$, $p<0.01$) (figure 1).

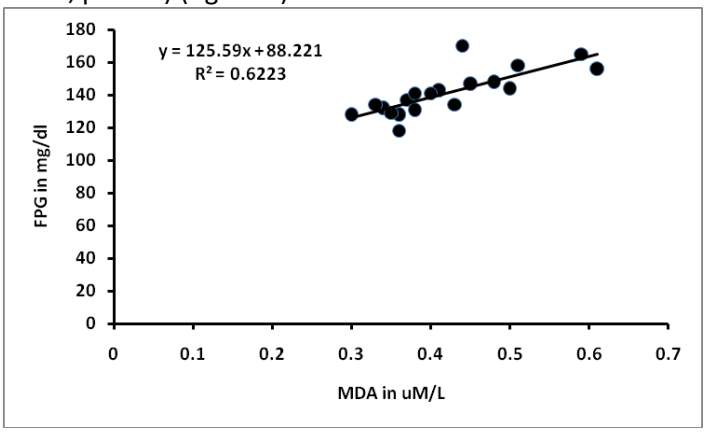

Figure1. Correlation between MDA and FBG in schizophrenic patients with diabetes mellitus.

	Controls (N = 50)	Group I (N = 39)	Group II (N=21)
MDA (µM/L)	0.23 ± 0.04	0.30 ± 0.20*	0.41 ± 0.08#
Thiols (µM/L)	354 ± 57	300 ± 97*	242 ± 80#
Fasting Plasma Glucose (mg/dL)	101±4	104±5	140 ± 13#
Serum Urea (mg/dL)	28±5	21±8	30±10
Serum Creatinine (mg/dL)	0.83±0.2	0.84±0.14	0.9±0.13
Serum Aspartate transaminase (U/L)	16 ± 4	29±24	33±10
Serum Alanine transaminase (U/L)	18 ± 4	20±9	29±9
Total bilirubin (mg/dL)	0.80±0.3	0.75±0.04	0.85±0.05
Direct bilirubin (mg/dL)	0.20±0.16	0.26±0.02	0.31±0.02

*p <0.001 compared to healthy controls.
#p <0.001 compared to group I and healthy controls

Table 1. Serum thiols and MDA levels in patients with schizophrenia compared to healthy controls. (Values are expressed in mean ± SEM)

Discussion

In line with previous studies we have found significantly elevated serum MDA levels in schizophrenic patients compared to healthy controls indicating increased presence of oxidant damage to biological membranes including neuronal cells. Oxidative stress had been attributed to pathology of most of the neurological disorders. The brain tissue contain large amounts of polyunsaturated fatty acids and are vulnerable to free radical induced lipid peroxidation which will generate MDA; considered as specific and sensitive marker of lipid peroxidation. (16)

We have found significant increase in MDA in schizophrenic patients with diabetes mellitus compared to schizophrenic patient without diabetes mellitus and healthy controls. In patients with diabetes mellitus, hyperglycemia itself can induce oxidative stress and also deplete antioxidants which in turn cause peroxidative membrane damage. (17) The positive correlation between MDA and FPG in schizophrenic patients with diabetes mellitus indicates more severe is the diabetes mellitus more will be membrane damage. Many of previous studies have shown that there is increased risk of diabetes mellitus in schizophrenic patients and which has been attributed due to disease itself, genetic factors, antipsychotic medications, life style changes and obesity. (18) According to previous studies there is increased risk of schizophrenia in babies born to diabetic mother. They proposed maternal hyperglycemia can predispose to schizophrenia in adult life through three prenatal mechanisms: hypoxia, oxidative stress and increased inflammation. Thus diabetes mellitus and schizophrenia are disease of adulthood with origins possibly outlined at birth. Considering the co-morbidity and close associations of these two diseases is mainly at genetic level as they carry common genetic elements.(19) According to some studies diabetes mellitus and schizophrenia share common susceptibility genes, in particular 2 genetic loci associated with schizophrenia have also been implicated in the linkage studies of patients with diabetes mellitus.(20)

In the present study, we have found decrease in total thiol levels in schizophrenia patients with diabetes mellitus compared to schizophrenic patients without diabetes mellitus and healthy controls. Protein bound thiols (-SH) groups are the abundant antioxidants in the body and has been shown to participate in various reductive reactions. The negative correlation between total thiols and MDA indicates the role of thiols in neutralizing reactive free radicals, and thereby preventing oxidative membrane damage. Previous studies have reported the dysregulation

of reduced glutathione (GSH) metabolism is one of the vulnerability factors contributing to development of schizophrenia. This clearly indicates the role of GSH in protecting nervous tissue against free radical induced membrane damage in neuronal tissues.

In conclusion, there is increased oxidative membrane damage in schizophrenic patients and which is further enhanced in schizophrenics with diabetes mellitus. This may be due to additional oxidative stress due to diabetes mellitus itself adding with oxidative stress of schizophrenia.

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Conflict of Interest: None Declared

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