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Original Article

Biochemistry Section

Plasma Total Thiols and Total Thiol/Albumin Ratio in Patients Suffering from Depression

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ABSTRACT

Introduction: Increased oxidative stress has been reported in patients who suffer from depressive disorders. Albumin acts as a target for plasma protein oxidation during oxidative stress. The plasma thiols act as significant in-vivo antioxidants. Major SH-groups are found on the surface of albumin molecules. Since depressive disorders are related to oxidative stress. Only a few studies have been done that correlate plasma total thiols with major depressive disorder. The authors intended to draw a relationship between oxidative stress, thiols and major depressive disorder by estimating total plasma thiols and calculating the ratio of thiol/albumin. The study may throw some light in understanding whether the use of anti-oxidant supplements to counter oxidative stress in depressed patients.

Aim: To estimate plasma thiols, albumin and obtain a plasma thiol/albumin ratio in people suffering from depression and compare the levels with the control group.

Materials and Methods: This case control study was conducted in Kasturba Medical College, Manipal. Plasma thiols were estimated using Ellman's method. Plasma albumin levels were estimated using Bromo cresol green dye binding method. Mann-Whitney U test was used for analysing the data for total thiols and thiol/albumin ratio. A p-value <0.05 was considered significant.

Results: The study group was made up of 43 (22 males and 21 females) patients and the control group was made up of 40 (18 males and 22 females) healthy controls. Plasma thiol levels and the plasma total thiol/albumin ratio were significantly elevated (p<0.001) in cases (depression) as compared to that of the controls.

Conclusion: Plasma total thiols can be used as an early marker for understanding the risk for major depressive disorders and also be used as a prognostic indicator in the follow-up of patients suffering from major depressive disorder who are under treatment.

Keywords: Major depressive disorder, Malon-di-aldehyde, Reactive oxygen species

INTRODUCTION

Elevated oxidative stress identified by elevated free radicals has been studied in patients with unipolar depressive disorder. As a common understanding, 12% of patients presenting to primary health care set up suffer from depression [1,2]. Antioxidant effects seen in antidepressants that are offered as a treatment of depression supports the concept that free radical stress may have a part in the pathology of depressive disorders. The stress may cause neuro-degeneration and lowered neurogenesis. There are data suggested that elevation in oxidative stress can be related to major depression and is proved by an elevated serum Malondialdehyde (MDA) levels and Super oxide dismutase (SOD) levels [3]. High levels of antioxidant enzymatic activity (SOD and glutathione peroxidase) have been found in patients with depression [4]. Albumin gets to be a target during oxidative stress for oxidation of plasma proteins. Since thiols are found to exhibit in-vivo anti-oxidant properties and the major free sulphydryl groups are present over albumin [4,5] the study was intended to estimate the same and correlate them with the depressive disorders. The study can help us to understand if in future anti-oxidants or the drugs that can increase total thiols, may be helpful in the treatment of depression.

MATERIALS AND METHODS

This was a case-control study conducted between September 2010 and September 2012.

The study was conducted at:

1. Departments of Biochemistry and Psychiatry, Kasturba Medical College and Hospital.
2. Department of Psychiatry, Dr. A. V. Baliga Memorial Hospital, Udupi.

After obtaining the approval from the Institutional Ethics Committee (Approval number: I.E.C./44/2011). of Kasturba Hospital, Manipal and the Director of Dr. A. V. Baliga Hospital, Udupi, study has been done in accordance with Helsinki guidelines for human studies. The study was conducted after getting the proper informed written consent of the cases' legal guardians and healthy controls.

Inclusion criteria: Cases: Patients of both sexes of age group 30 to 70 years, who were freshly diagnosed clinically as suffering from depressive disorders and not on any treatment for depressive disorders.

Controls: Healthy subjects of both sexes of age group 30 to 70 years, who have scored less than four major points (or) fourteen minor points after answering the Goldberg's general health questionnaire-28 [6].

Exclusion criteria: Subjects less than 30 years and more than 70 years of age/those suffering from depression but having symptoms of psychosis/with disorders suggestive of psychosis (schizophrenia)/ tuberculosis/diabetes mellitus/Acquired Immunodeficiency Syndrome (AIDS)/thyroid gland based illness/seizure disorders/long standing defects in kidney, liver or brain function and any chronic illnesses and subjects who were on treatment for any of the above conditions. Subjects who were chronic alcohol and nicotine consumers were excluded too.

Serum Separation

Samples were collected by venepuncture into the Lithium heparin containers in the fasting period from each patient before admission.

Blood was allowed to clot in an upright position for atleast 30 minutes but not longer than one hour before centrifugation. Centrifugation was done for atleast 15 minutes at 2200-2500 RPM within one hour of collection.

Assay of Plasma Protein Thiols

Method: Ellman's method [7-9]

Free thiols were quantified using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) a chromogenic reagent. DTNB has an oxidising disulfide

bond which in the presence of free thiols is reduced forming a mixed disulfide and releases one molecule of 2-nitro 5-thio benzoic acid (TNB). Free thiol concentration is estimated by observing absorption at 412 nm and measuring TNB was measured.

Assay of Plasma Albumin

Method: BCG-dye binding method [10]

When albumin is added to a solution of Bromo cresol Green dye (BCG), an anionic dye in a succinate buffer of pH 4.2 the binding of albumin and BCG results in a color change of the indicator from yellowish green to greenish blue with an increase in absorbance at 628 nm. The absorbance concentration relationship is linear for samples containing up to 6 g/dL albumin.

STATISTICAL ANALYSIS

Statistical Package for the Social Science (SPSS) software version 16.0 was used to analyse the data after estimating plasma total thiols and calculating plasma total thiol/albumin ratio in both the subjects-controls and cases. The data was found to be non-normal in the way it was distributed. So, non-parametric Mann-Whitney U test was used. A p-value <0.05 were considered significant.

RESULTS

The total number of participants in the study was 83. Out of them, 43 were cases and 40 were healthy controls. Of the 43 cases, 22 were males and 21 were females. Of the 40 controls, 18 were males and 22 were females. The total thiols results are presented in [Table/Fig-1]. There was a significant increase in the plasma total thiols in cases compared to controls. There was a significant increase in the thiol to albumin ratio in the cases compared to the control groups.

Parameter	Controls (n=40)	Cases (n=43)	*p-value
Plasma total thiols (µmol/L) Median (Inter quartile range)	139.5 (78.07-291.38)	245.5 (191-342)	p<0.001
Plasma total thiol/albumin ratio Median (Inter quartile range)	3.99 (2.37,8.16)	6.81 (4.58,9.13)	p<0.001

[Table/Fig-1]: Comparison of plasma total thiols and total thiol/albumin ratio between cases and healthy controls.
*Mann-Whitney U test; p-value <0.05 considered significant

DISCUSSION

Depressive disorders affect mentation and mood that negatively impacts cognition and the quality of life. Increased lipid peroxidation has been studied in patients with Major Depressive Disorder (MDD) which also suggest may be normalised by anti-depressants. Studies have shown a strong relationship with oxidative stress and patho physiology of depressive disorders affecting the size/volume of hippocampus of patients [1,2,5,11]. This is understood to be a result of cell apoptosis triggered by oxidative stress [4].

Reactive oxygen species can structurally and functionally disturb lipids, Deoxyribonucleic Acid (DNA) and proteins. This disturbance toxically injures the neurons [5]. MDD is understood as a neuro-progressive process, and may involve structural brain consequences. It has a possible role of inflammatory mediators, nitrosative stress pathways, and regulation of neurogenesis [12]. When the factors that encourage oxidation are more in content than those that reduce oxidation in-vivo, then the state is referred to as oxidative stress. The state leads to modification of normal cellular proteins like protein thiols and glutathione causing modified end products of oxidation and per-oxidation to enter circulation. MDA in the serum is a product of lipid peroxidation whose rise in patients with depressive disorder indicates oxidative stress [13].

A meta-analysis study on MDD has shown a significant rise in markers of lipid per-oxidation. It has also showed a direct relationship between the severities of depressive disorders with the degree of lipid peroxidation [14]. Previous data have clearly shown that, treatment with antidepressants caused a significant reduction in concentrations of lipid peroxidation markers in patients with MDD [14]. This supports the concept of oxidative stress in general and lipid peroxidation in particular has a role in MDD. Boosting anti-oxidant defenses can be one of the possible explanations for the neuro-protective effects of antidepressants in the treatment of MDD [4]. Further studies on oxidative stress to lipids to understand the patho physiology of MDD may be needed and it may be conducted on antidepressant-naive individuals.

In plasma, the thiol groups (free thiols) by sheer numbers act as significant scavengers of free radicals like hypochlorite radicals and a few oxidants who are seen on the surface of albumin molecules [15]. A one such example of a free radical is a superoxide free radical now considered as a carefully regulated metabolite involved in communicating with cell's genetic machinery and possibly signaling. Gene expression is redox regulated by superoxide and other related oxidants. These concepts are emerging as vital mechanisms in health and disease [16]. Using those thiol groups, albumin provides an approximately ten times higher antioxidant protection against the above mentioned free radicals in plasma [17-20].

In this present study, the plasma total thiol levels and the plasma total thiol/albumin ratio were significantly elevated (p-value <0.001) in cases (depression) as compared to that of the controls. Some anti-oxidant enzymes get up-regulated during oxidative stress [11]. Data have shown elevated level of plasma thiols which were induced by increased oxidative stress. Some other studies have shown a fall in thiol levels during the first episode of depression before treatment [21]. Recent studies could not show a clear relation between oxidative stress and major depressive disorders, some have found an elevation in the thiol protein groups in depression and cognitive impairment [21,22]. This finding was in contrast to the present study finding and might be because of the consumption of the thiols in an attempt to reduce the oxidant molecules. Thiol/disulfide homeostasis may be useful as a biomarker for depression after long-term follow-up and treatment studies. The studies on effect on anti-depressants can further the cause of investigating usage of drugs and anti-oxidants to control episodes of depression or alter the outcomes of the same.

The findings in this study suggested that oxidative stress as understood by elevation of the thiol levels and the thiol/albumin ratio is significantly related to patients suffering from depressive disorders.

Limitation(s)

Size of the sample in this study was relatively small owing to the number of MDD patients attending the clinic who offered consent for the study. This can be a limitation of this study. Other markers of oxidative stress could have been estimated and correlated with plasma total thiols and plasma total thiol: albumin ratio.

CONCLUSION(S)

This study shows that patients with MDD have elevated levels of total plasma thiols indicating that the rise is a response to the underlying oxidative stress. Previously only few other studies have not shown such a statistically significant elevation. Oxidative stress in the initial untreated stages may show either a fall or a rise in thiol levels depending on newly synthesised thiols or the exhaustion of the same in the fight against free radicals. Some studies showing anti-depressants to have a fall in oxidant stress reinforces the relationship between oxidant stress and MDD. The study population was the patients with MDD visiting the psychiatric OPD and hence the size of the same limited. Further studies on the same can throw more light on how the stress markers may be diagnostic and prognostic in the MDD.

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