



“STUDY OF ASSOCIATION OF OXIDATIVE STRESS AND INFLAMMATION IN NEUROPSYCHIATRIC DISORDERS”

Dr. Santoshi R. Ghodake^{*1,2} and Dr. Adinath N. Suryakar^{1,3}

¹UDIRT, Maharashtra University of Health Sciences, Nashik, Maharashtra, India.

²Saraswati-Dhanwantari Dental College and Hospital, Parbhani, Maharashtra, India.

³Dr. D Y Patil University, Pune, Maharashtra, India.

*Corresponding Author: Dr. Santoshi R. Ghodake

UDIRT, Maharashtra University of Health Sciences, Nashik, Maharashtra, India.

Article Received on 26/05/2017

Article Revised on 16/06/2017

Article Accepted on 06/07/2017

ABSTRACT

Objective: Neuropsychiatric disorders represent the second largest cause of morbidity worldwide having very complex etiopathophysiology. Several lines of evidence suggesting that inflammatory reactions are most common features of all forms of neuronal disorders. There is growing evidences also suggests increased oxidative stress and inflammation in patients with schizophrenia. To assess the lipid peroxidation, Malondialdehyde (MDA), Total antioxidant capacity (TAC) uric acid were estimated. To estimate the activity of Adenosine deaminase (ADA) as inflammatory marker in neuropsychiatric disorders. Also study was aimed to assess the impact of antioxidant supplementation on these parameters in patients with neuropsychiatric disorders. **Method:** The study group included a total of 90 subjects of which 30 were schizophrenic patients, 30 were major depressive patients and 30 were healthy controls. Statistical analysis was performed using student 't' test. **Results:** Serum MDA (Group I, $p < 0.0001$ Group II, $p < 0.05$) and ADA (Group I, $p < 0.0001$, Group II, $p < 0.05$) levels were found to be significantly high in both group patients (schizophrenics and depression) when compared to controls. Significant decreased levels of TAC (Group I and II, $p < 0.0001$) and uric acid (Group I and II, $p < 0.05$) level in were observed in both groups. After antioxidants supplementation significant changes were found in the levels of MDA, TAC, uric acid and ADA activity. **Conclusion:** We conclude that increased serum ADA activity indicates inflammation and increased MDA level indicates oxidative stress in neuropsychiatric disorders, schizophrenia and depression. The data showed that decrease in plasma TAC and uric acid, suggesting increased oxidative stress. Inflammatory biomarkers are known to play an important role in initiation and progression of disease along with oxidative damage.

KEYWORDS: Adenosine deaminase, Malondialdehyde, Oxidative stress, Inflammation, Neuropsychiatric disorders.

INTRODUCTION

Neuropsychiatric disorders are debilitating conditions associated with excess and premature mortality and are a great burden on society where there is currently no approved treatment to prevent these diseases from affecting patients.^[1] Disorders such as depression, schizophrenia are increasingly affecting societies worldwide. Clinical schizophrenia is connected with a number of vegetative, psychological and biochemical processes in a human organism. Major depressive disorder (MDD) is associated with a significantly increased risk of developing serious medical illnesses that are more commonly seen with advanced age, such as diabetes, cardiovascular disease, immune impairments, stroke, dementia, osteoporosis, diabetes and metabolic syndrome. Neuronal deterioration following stroke, often display co-morbidity with neuropsychiatric symptoms

and behavioral alterations. These include depressive illness and delusion characteristic of schizophrenia.^[2,3]

The adult human central nervous system (CNS) consists of approximately 100 billion neurons and a similar amount of glia cells, namely, astrocytes, oligodendrocytes and microglia. Neural cells are considered to be more susceptible to oxidative damage as compared to other body tissues. The CNS parenchyma is separated from the rest of the body by the blood-brain barrier (BBB), which is formed predominantly by tight junctions of the endothelial cells of the CNS vasculature. The BBB restricts and controls the entry of nutrients and cells, including peripheral immune cells, which are almost completely absent in the healthy CNS. This has led to the concept that the CNS is an immune privileged organ. However, this concept has been modified in recent years since the CNS itself is fully immune

competent and quickly responds to injury or infections.^[4,5]

Now it was generally accepted that oxidative stress contributes to the pathogenesis of neuropsychiatric disorders. Recent findings suggest that neuroinflammation is an important player in the pathophysiology of neuropsychiatric diseases such as stroke, depression, Alzheimer's diseases, or schizophrenia.^[4] Numerous studies support an interdependent relationship between inflammation and oxidative stress. Inflammation and oxidative stress are closely related and tightly linked processes. Chronic inflammation is mostly associated with levels of elevated reactive oxygen species (ROS); an anti-inflammatory cascade is linked to the process of scavenging ROS concentrations. Thus, both processes are simultaneously found in many pathological conditions.^[4,5]

It is not clear whether increase of reactive oxygen species (ROS) is the cause or consequence of neuropsychiatric disorders. Production of pro-inflammatory cytokines is increased and inflammatory response system is activated in depressive disorders and schizophrenia.^[3] Recent studies suggest that following stress, tissues initiate an inflammatory cascade that includes acute phase protein synthesis, up-regulation of inflammatory adhesion cell molecules and pro-inflammatory cytokine release.^[6,7]

Lipid peroxidation may increase in conditions with psychological stress such as depression. There has also been some evidence that the antioxidant system is impaired in schizophrenia.^[8] There are few literature bases to suggest that adenosine plays a role in the regulation of inflammatory diseases including neuropsychiatric diseases.^[9]

Malondialdehyde (MDA) is a secondary lipid peroxidation end product and is specific marker of lipid peroxidation of membrane.^[10] In neuropsychiatric disorders, the serum level of MDA is increased and total antioxidant capacity (TAC) is decreased, which indicate the oxidative stress. TAC is a useful index for the measurement of the activity of antioxidants in plasma.^[11] Recent studies indicate that equilibrium between oxidant and antioxidant systems is impaired in schizophrenia and depressive patients.^[12,13,14] It was also suggested that there is a direct relationship between oxidative stress and the serum adenosine deaminase (ADA) activity as an inflammatory marker.^[15]

ADA, adenosine amino hydrolase is an enzyme present in all nucleated cells predominantly T cells, involved in the metabolism of purine bases, catalyzing the deamination of adenosine, forming inosine. Its main physiological activity is related to lymphocytic proliferation and differentiation. As a marker of cell mediated immunity, its activity is found to be elevated in those diseases in which there is a cell – mediated

immune response.^[16] Since, adenosine modulates the release of several neurotransmitters such as glutamate, dopamine, serotonin and acetylcholine, decreases neuronal activity by post-synaptic hyper-polarization and inhibits dopaminergic activity adenosine metabolism in the brain is very important.^[16,17] ADA activity is known to be increased in inflammatory diseases characterized by T-cell activation and proliferation. Therefore, ADA is considered a marker of T-cell activation.^[15,16] Uric acid is a diprotic acid. It is a product of the metabolic breakdown of purine nucleotides. Serum uric acid is one of the important contributors of antioxidant capacity. When the uric acid level is elevated, it functions as pro-oxidant rather than antioxidant. The antioxidant and oxidant function of uric acid mainly depends on various factors such as depletion of antioxidants, surrounding oxidant environment, acidity etc.^[15,17] Recent studies found that there is a definite relationship between the activity of the ADA and end product of purine catabolism uric acid. Study also indicated the relationship between the oxidative stress and the serum uric acid level, indicating that serum uric acid acts as pro-oxidant.^[17]

Recent data suggest that antioxidant vitamin therapy can provide tissue protection by inhibiting translocation of the transcription factor NF- κ B and interrupting the secretion of inflammatory cytokines.^[3,4,18] Vitamins E and C are scavengers of free radicals and play important roles, beyond their antioxidant properties, in cell function. These nutrients provide reducing equivalents within the cell for critical enzymatic functions and may be most important in oxidative injury following glial cell activation in the brain and/or reducing the oxidative-mediated damage; the latter may be relevant to ameliorate or delay the damage caused by inflammatory processes in neuronal cells. These two vitamins may therefore have important effects on the rate of progression of neurodegenerative disease and on cognitive performance.^[4,7,19]

Taken together, these data suggest that ADA, uric acid can acts as markers of inflammation as well as oxidative stress in the pathophysiology of neuropsychiatric disorders. The present study was therefore undertaken to examine whether the interdependence between oxidative stress and inflammation. In the present study an attempt to estimate the levels of serum ADA activity and uric acid levels besides the oxidant stress index MDA and TAC in neuropsychiatric patients. This study was also aimed to explore the beneficial effects of combined supplementation of antioxidants (vitamin E and C) on oxidative stress and inflammatory parameters in neuropsychiatric patients.

MATERIALS AND METHODS

The study was approved by University ethical and review board for the patients and healthy control subjects. Patients were consecutively recruited from outpatients or inpatients of the department of Psychiatry.

All subjects provided written informed consent (from patient's relatives). The patients group was composed of 30 schizophrenic patients (Group I) and 30 patients with major depression (Group II) (age range 19-55 years) diagnosed according to DSM-IV (American Psychiatric Association Diagnostic and Statistical Manual of Mental disorders) criteria.^[20] 30 normal healthy control volunteers of matched age and sex were recruited to participate (included in the study for comparison).

Exclusion criteria

The following exclusion criteria for patients and the control group were applied:

The patients and controls none had a history of any cardiovascular or neurological or drug or alcohol abuse. No patient was being treated with antipsychotic or antidepressant medications. Not having any somatic disorders, especially, disorders of lipid metabolism and diabetes mellitus, malnutrition, obesity and other neurological disorders and serious head injuries. The subject had normal Body Mass Index (BMI) did not use any addictive substances or antioxidant supplementation. Their diet was balanced. Heavy smokers were excluded from both the group. All subjects were not showing any abnormalities of immunoresponse.

Blood sampling / collection and measurements

The fasting blood samples were collected in heparinized bulb (3 ml) and plain bulb (2 ml) on the initial test day. Plasma and serum was separated by centrifugation at 3000 g for 15 minutes. Separated plasma and serum were used for the estimation of MDA, TAC and ADA and uric acid respectively. For estimating serum MDA, an indicator of oxidative stress, measuring and a secondary fragmentation product of PUFA (polyunsaturated fatty acid) peroxide, a thiobarbituric acid- reactive substance (TBARS) that gives a pink color complex with TBA. It was read on a spectrophotometer at 535 nm wavelength.^[21] Plasma TAC was measured by the assay of FRAP.^[22] Serum ADA activity was assayed by Giusti and Galanti method^[23] and Serum Uric Acid level by uricase method using kit.^[24] All the reagents used were of analytical reagent grade. All parameters were assessed

again after antioxidant supplementation along with routine antipsychotic/ antidepressant therapy. The supplementation was done with vitamin E (400IU/ day) and vitamin C (250mg/day) for 20 weeks (5 months). The overall study was carried out in accordance with Helsinki declaration made in 1975 (revised in 2000).

Statistical analyses

Statistical analysis between controls and patients was performed by students't' test using Graph Pad Prism, Version 3.02 software. The data were expressed as mean $\pm$ SD, $p < 0.05$ was considered as significant.

RESULTS

A total of 90 subjects were recruited for the study. Among them, 30 were schizophrenic patients (Group I), 30 belonged to patients with major depression (Group II) and 30 belonged to the healthy controls. Analysis of mean values of the parameters for oxidative stress and antioxidant status between pre and post supplementation in both patient groups are shown in the Table 1. Student t test had demonstrated that in all the neuropsychiatric patients, MDA levels in plasma were significantly raised in both group patients (Group I, $p < 0.05$ and Group II, $p < 0.0001$) when compared to that of normal healthy control individuals. However, the mean plasma level was found to be significantly decreased (Group I, $p < 0.0001$, Group II, $p < 0.05$) after supplementation. TAC levels were also found to be increased significantly (Group I and Group II, $p < 0.0001$) after supplementation. Plasma TAC in all patients before supplementation was significantly lower (Group I, $p < 0.05$ and Group II, $p < 0.0001$) that the controls group.

Serum ADA levels were significantly elevated (Group I; $p < 0.0001$, Group II; $p < 0.05$) in both patients groups and serum uric acid levels were significantly (Group I and Group II; $p < 0.05$) decreased in patients when compared to healthy controls. After antioxidants supplementation serum ADA activity reduced significantly (Group I; $p < 0.0001$, Group II; $p < 0.05$) and uric acid levels were significantly increased (Group I and Group II, $p < 0.05$).

Table 1. Comparison of levels of MDA, TAC, ADA activity and Uric acid among healthy control subjects, schizophrenic and major depressive patients.

Parameters	Controls	Group I (Schizophrenic patients)		Group II (Major Depression)	
		Before supplementation	After supplementation	Before supplementation	After supplementation
MDA (nmol/dl)	260.82 ± 26.10	352.83 $\pm 45.96^{***}$	272.30 $\pm 45.01^{***}$	295.28 $\pm 65.92^*$	260.18 $\pm 30.83^*$
TAC (μ mol/L)	965.80 ± 161.94	731.03 $\pm 128.09^{***}$	825.00 $\pm 86.85^*$	720.50 $\pm 125.20^{***}$	838.17 $\pm 91.13^{***}$
ADA (U/L)	16.16 ± 2.67	24.85 $\pm 2.53^{***}$	17.38 $\pm 2.18^{***}$	19.52 $\pm 5.00^*$	16.73 $\pm 4.81^*$
Uric acid(mg/dl)	4.45 ± 1.06	3.64 $\pm 1.21^*$	4.22 $\pm 1.07^*$	3.80 $\pm 1.06^*$	4.30 $\pm 1.12^*$

All values are expressed as Mean $\pm$ SD

n=Indicates the no. of subjects

* Comparison between controls and patients-Before supplementation (unpaired 't' test),

• Comparison between before supplementation and after supplementation (paired 't' test)

***, **p<0.0001=Very Highly significant, *, •p< 0.05= Significant, p>0.05=Non-Significant

DISCUSSION

Increased oxidative stress and inflammatory biomarkers are known to play an important role in the initiation and progression of neuropsychiatric disorders.^[25,26,27] In the present study, variations in the levels of MDA, TAC, ADA activity and uric acid level in neuropsychiatric patients (schizophrenia and depression) from that of the healthy controls have been evaluated. In present study it was observed that the levels of MDA were significantly increased in both patient groups compared to healthy controls. Increased levels may be attributed to increased membrane peroxidation in CNS detected peripherally due to a loss of blood brain barrier.^[6] Lipid peroxidation is an index of irreversible neuronal damage which suggested the possible mechanism of pathogenic activity in neuropsychiatric disorders.^[25]

Activation of oligodendrocytes results in secretion of inflammatory molecules, such as nitric oxide (NO), cytokines and prostaglandins and thus, pro-inflammatory response system is activated. This process may lead to an increase in lipid peroxidation.^[7]

TAC is a useful index for the measurement of the activity of antioxidants in a medium. Our presented results demonstrates that the significantly lower plasma TAC (p<0.0001) in both patient groups than that of the control group, together suggests that levels of antioxidants in plasma are decreased. Studies, analyzing antioxidant substances or the integrated plasma total antioxidant activity in patients showed same results.^[11,12,13,14,28,29] Our observation is in agreement with published studies documenting low total antioxidant capacity.

In this regard in order to make a contribution to the understanding of the ongoing immune disturbance in neuropsychiatric disorders, serum ADA activity was determined in neuropsychiatric patients and compared with healthy controls. Intracellular and extracellular levels of adenosine are tightly controlled by specific nucleoside transporters and several important enzymes which including ADA.^[16]

ADA can be considered as an index for developing inflammation. It has strongly been suggested that serum ADA activity reflects monocyte/ macrophage activity or turnover in different diseases.^[30,31] In present study, serum ADA activity was also found to be high, suggests that there might be a disturbance related to cellular immune system functioning in neuropsychiatric disorders.^[5]

The ADA activity significantly above the statistical mean value, the development of severe inflammation leads to the ADA release because of tissue destruction and cell

death. But the ADA release by itself might be the cause of severity increase of inflammation, as it metabolizes and deactivates a very important anti-inflammatory mediator adenosine.^[17,32]

Increased activity of ADA can increases the xanthine oxidase substrate formation leads to excess of free radical generation. In reperfused tissues, xanthine oxidase in the presence of its substrate hypoxanthine or xanthine reduces molecular oxygen to $\bullet\text{O}_2^-$ and H_2O_2 , which can further react to form the more reactive $\bullet\text{OH}$.^[12,13] The $\bullet\text{OH}$ and $\bullet\text{O}_2^-$ radicals produced by the enzyme can then in turn oxidize cellular proteins and membranes resulting in cellular injury.^[33,34]

Oxidative damage triggers inflammatory reactions, whereas the cellular response for redox balance is inhibited. Redox-mediated increase in free radicals may leads to augmented expression of several pro-inflammatory genes whose expression is mediated through the transcription factor nuclear factor-kappa-B or NF- κB .^[6,7] Inducible nitric oxide synthase (iNOS) and cyclo-oxygenase type 2 (COX-2) enzymes, which we know helps convert arachidonic acid (AA) into inflammatory prostaglandins. Similarly, the up-regulation of NADPH-oxidase, which is known to be associated with inflammation and ROS generation, has been tied to microglial activation, local ROS elevation, and subsequent neuronal loss. In addition, overproduction of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion ($\text{O}_2^{\bullet -}$); nitric oxide ($\text{NO}^\bullet$) and singlet oxygen (O_2) creates a condition known as oxidative stress, resulting in the amplification of the inflammatory response.^[6,8,34]

Studies related to ADA levels in neuropsychiatric patients are virtually existed very rare. **Dutra GD et al (2009)** showed that decreased ADA activity in schizophrenic patients than in control.^[32] **Brunstein MG et al (2007)** reported that the schizophrenic patients treated either with typical antipsychotics or clozapine showed increased serum ADA activity compared to controls.^[33] **Herken H et al (2007)** showed that ADA activity of the patients were significantly higher than the controls.^[34] **Elgun et al (1999b)** reported that decrease ADA activity in patients with depression compared with controls.^[35]

We have also estimated the levels of uric acid which is a protective antioxidant, particularly effective in quenching hydroxyl, superoxide and peroxynitrite radical, thereby preventing lipid peroxidation. Though we expect an increase in uric acid levels due to increase in ADA levels in patients, our study showed decreased uric acid levels. Few studies which also showed decreased uric acid levels in the schizophrenic and

depressed patients. This is probably may be due to increased utilization of uric acid in trapping the free radicals produced and its conversion to allantoin.^[15,17,36]

Supplementation of non-enzymatic antioxidant compounds, including vitamin E, and C as an addition to the treatment of schizophrenia and depression, may protect membranous structures from lipid peroxidation by ROS and RNS (reactive nitrogen species).^[11,12,25] Beside antioxidant properties, vitamin C and E have been shown to interact with the brain's catecholamines. In these two different ways, it might be possible to produce a remarkable improvement in some symptoms of schizophrenia.^[25,37]

In present study, after antioxidants (vitamin E and C) supplementation showed significant changes in the levels of all parameters. Serum MDA levels become significantly decreased where as TAC becomes increased to significant extent. Inflammatory marker ADA activity was significantly decreased and uric acid levels increased to normal after antioxidants supplementation.

Antioxidant vitamins may stop ongoing oxidative damage and have a potential to restore the cellular structure their combined use may be necessary for optimal treatment of oxidative cell damage.^[7,10] Other proposed mechanisms by which increased intake and plasma concentrations of the antioxidants E and C may positively influence the development of pathologic disorders including maintaining the immune system (enhances lymphocyte proliferation and decreases production of immunosuppressive prostaglandin E2), platelet function (inhibits platelet adhesion), formation and repair of collagen and muscle and brain function.^[19,25] But, some studies proposed that antioxidant therapy alone is unlikely to prevent diseases known to be induced by oxidative stress, like cardiovascular and diabetic complications, neurodegenerative diseases, cancer, or aging.^[7] Interestingly, our present results indicated that antioxidant vitamin E and C supplementation showed beneficial effects in minimizing oxidative stress and inflammatory markers which may helpful for slowing of the disease process.

CONCLUSION

The present study concluded the association of oxidative stress and inflammation in neuronal damage leading to disease pathogenesis. Two main aspects contribute to the vulnerability of the CNS to oxidative stress mediated neuronal damage: high metabolism and restricted cell renewal. The CNS is a metabolically highly active organ, requiring approximately 20% of the total energy consumption of the body. Therefore the CNS contains high amounts of mitochondria, which are particularly active, resulting in high amounts of ROS. Again CNS is an immune privileged organ. In present study, serum adenosine ADA activity has been found to be increased in both patient groups, which indicates activation of cell

mediated immunity. Increase in the level of MDA and decreased in levels of TAC and uric acid patients indicates the important role played by ROS, in tissue damage and inflammatory consequences in this disorder. After vitamins supplementation the levels of MDA and ADA activity decreased significantly and the significant change was observed in the levels of TAC and uric acid. Perhaps, reduction in lipid peroxidation and supplementation with antioxidants vitamin E and C may positively influence the development of pathologic disorders including maintaining the immune system along with returning antioxidant balance to normal state.

Further studies on inflammatory markers and its association with oxidative stress in neuropsychiatric disorders are still necessary to improve our understanding of the disease pathogenesis.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

BIBLIOGRAPHY

1. Oksana KB, Danielle SC and Roger SM. Crosstalk between metabolic and neuropsychiatric disorders. *F1000 Biology Reports*, 2012; 4: 14.
2. Elena IK, Bashkim M, Elena VD, Mariya VB, Evgeniy VP and Wolfgang L. Study of OH• radicals in human serum blood of healthy individuals and those with pathological schizophrenia. *International Journal Molecular Sciences*. 2011; 12: 401-409.
3. Wolkowitz OM, Mellon SH, Epel ES, Lin J, Dhabhar FS, et al. (2011) Leukocyte Telomere Length in Major Depression: Correlations with Chronicity, Inflammation and Oxidative Stress - Preliminary Findings. *PLoS ONE* 6(3): e17837.
4. Fischer R and Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxidative Medicine and Cellular Longevity* 2015; Article ID 610813:18 pages.
5. Tansey MG. Inflammation in neuropsychiatric disease. *Neurobiol Dis*. 2010; 37(3): 491-492.
6. Popa-Wagner A, Mitran S, Sivanesan S, Chang E and Buga AM. ROS and brain diseases: the good, the bad, and the ugly. *Oxidative Medicine and Cellular Longevity* 2013; Article ID 963520, 14 pages.
7. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative Medicine and Cellular Longevity* 2016; Article ID 5698931, 9 pages.
8. Yegin A, Ay N, Aydin O, Yargici N, Eren E and Yilmaz N. Increased Oxidant Stress and Inflammation in patients with chronic schizophrenia.

- International Journal of Clinical Medicine 2012; 3: 368-376.
9. Chunn JL, Young HWJ, Banerjee SK, Colasurdo GN et al. Adenosine-dependent airway inflammation and hyperresponsiveness in partially adenosine deaminase-deficient mice¹. The Journal of Immunology 2001; 167: 4676-85.
 10. Niki E, Yoshida Y, Saito Y and Noguchi N. Lipid peroxidation: Mechanisms, inhibition, and biological effects. Biochemical and Biophysical Research Communications 2005; 338: 668-676.
 11. Cumurcu BE, Ozyurt H, Etikan I, Demir S and Karlidag R. Total antioxidant capacity and total oxidant status in patients with major depression: Impact of antidepressant treatment. Psychiatry and Clinical Neurosciences 2009; 63: 639-645.
 12. Yapişlar H. Erythrocyte deformability and antioxidant status in schizophrenia. Marmara Medical Journal 2012; 25: 133-7.
 13. Kuloglu M, Ustundag B, Atmaca M, Canatan H, Tezcan AE, Cinkilinc N. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Funct 2002c; 20: 171-5.
 14. Bal N, Acar ST, Aylin Yazıcı A, Kemal Yazıcı K et al. Altered levels of malondialdehyde and vitamin E in major depressive disorder and generalized anxiety disorder. Adam the Journal of Psychiatry and Neurological Sciences 2012; 25: 206-11.
 15. Sangeetha P, Vijayabanu N, Ponnudhali D and Jones E. Serum adenosine deaminase, uric acid and Malondialdehyde levels in rheumatoid arthritis. 2012; 3(2): 168-170.
 16. Ramkumar V, Hallam DM and Nie Z. Adenosine, oxidative stress and cytoprotection. Jpn J Pharmacol 2001; 86: 265- 74.
 17. Dasegowda SM, Jeppu AK, Kumar KA and Sushith S. Serum uric acid and its relation to adenosine deaminase, lipid profile and oxidative stress in Diabetes Mellitus type 2. J Invest Biochem 2016; 5(2): 32-36.
 18. Miller AH, Maletic V, and Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009 May 1; 65(9): 732-41.
 19. Martin A, Youdim K, Szprengiel A, Shukitt-Hale B et al. Roles of vitamins E and C on neurodegenerative diseases and cognitive performance. Nutrition Reviews 2002; 60(11): 308-34.
 20. American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Press, Washington 20.
 21. Bird RP and Draper HH. Comparative study on different methods of malondialdehyde determination. Methods Enzymol 1984; 105: 299-305.
 22. Benzie I, strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant Power": The FRAP Assay. Analytical Biochemistry. 1996; 239: 70-6.
 23. Giusti G and Galanti B. Adenosine deaminase: colorimetric method. Pp315-23 in HU Bergmeyer, methods of enzymatic analysis Vol 4, 3rd edition, Verlac Chemis Weinheim.
 24. Tietz N. W., Textbook of Clinical Chemistry, W. B. Saunders Company, Philadelphia, 1986.
 25. Zhang XY, Yao JK, Oxidative stress and therapeutic implications in psychiatric disorders, Prog Neuro-Psychopharmacol Biol Psychiatry 2013. <http://dx.doi.org/10.1016/j.pnpbp.2013.03.003>.
 26. Michel TM, Pulschen D and Thome J. The role of oxidative stress in depressive disorders. Current Pharmaceutical Design 2012; 18: 5890-9.
 27. Dietrich-Muszalska A and Kontek B. lipid peroxidation in patients with schizophrenia. Psychiatry and Clinical neurosciences 2010; 64: 469-75.
 28. Uma Devi P, Devipriya D, Chinnaswamy P. Age and gender related changes in total antioxidant response and oxidative stress in patients with schizophrenia. Journal of Clinical and Diagnostic Research 2008; 2(1): 627-33.
 29. Yanik M, Erel O, Kati M. The relationship between potency of oxidative stress and severity of depression. Acta Neuropsychiatrica 2004; 16: 200-3.
 30. Yarpuz AY, Yilmaz A, Soykan A, Elgun S et al. Levels of adenosine deaminase and dipeptidyl peptidase IV in patients with panic disorder. Turkish J of Psychiatry 2008; 19(2): 1-7.
 31. Erkilic K, Evereklioglu C, Cekmen M, Ozkiris A et al. Adenosine deaminase enzyme activity is increased and negatively correlates with catalase, superoxide dismutase and glutathione peroxidase in patients with Behcet's disease: original contributions/clinical and laboratory investigations. Mediators of Inflammation 2003; 12(2): 107-16.
 32. Dutra GP, Ottoni GL, Lara DR and Bogo MR. Lower frequency of the low activity adenosine deaminase allelic variant (ADA1*2) in schizophrenic patients. Revista Brasileira de Psiquiatria 2010; 32(3): 275-8.
 33. Brunstein MG, Silveira EM Jr, Chaves LS, Machado H, Schenkel O, Belmontede- Abreu P, Souza DO and Lara DR. Increased serum adenosine deaminase activity in schizophrenic receiving antipsychotic treatment. Neurosci Lett 2007; 414(1): 61-4.
 34. Herken H, Gurel A, Selek S, Armutcu F, Ozen ME, Bulut M, Kap O, Yumru M, Savas HA and Akyol O. Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. Archives of Medical Research 2007; 38: 247-52.
 35. Elgun S, Keskinoglu A and Kumbasar H. Dipeptidyl peptidase IV and adenosine deaminase activity, decrease in depression. Psychoneuroendocrinology 1999b; 24: 823-32.

36. Choudhari K, Khanzode S, Khanzode S, Dakhale G et al. Clinical correlation of alteration of endogenous antioxidant- uric acid level in major depressive disorder. *Indian Journal of Clinical Biochemistry* 2010; 25(1): 77-81.
37. Bayani U, Singh AV, Zamboni P and Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology* 2009; 7: 65-74.