ORIGINAL RESEARCH PAPER

Volume-8 | Issue-7 | July - 2019 | PRINT ISSN No. 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

THE ASSOCIATION URIC ACID AND ADENOSINE DEAMINASE IN NEUROPSYCHIATRIC DISORDERS.

Biochemistry

Dr. Santoshi R. Ghodake* Department of Biochemistry, Saraswati-Dhanwantari Dental College and Hospital, Parbhani, Maharashtra, India. *Corresponding Author

Dr. Adinath N. Suryakar Dr. DY Patil University, Pune, Maharashtra, India.

ABSTRACT

OBJECTIVES: Oxidative stress and inflammation may be seen as both causes and consequences of cellular pathophysiology in many disorders, including neuropsychiatric disorders. Uric acid (UA) and adenosine deaminase (ADA) levels are associated Oxidative stress and inflammation.

METHODS: The study group included a total of 480 subjects of which 240 were healthy controls and 240 of patients (60 schizophrenia, 60 major depression and 60 Epilepsy and 60 Alzheimer's disease.

RESULTS: Significant decreased in UA and significantly high MDA and ADA activity were found in all group patients when compared to controls. Significant negative correlations between the UA and ADA levels in all patient groups except epileptic patients (Group III).

Conclusion: It is possible that low serum UA levels are not able to prevent the free radical toxicity, leading to the development of inflammatory reactions leading to tissues destruction due to oxidative stress.

KEYWORDS

Oxidative stress, Inflammation, Uric acid Adenosine deaminase.

INTRODUCTION:

Neuropsychiatric disorders are debilitating clinical illness associated with enormous and premature mortality in patients. Oxidative stress and inflammation are inextricably related processes. Oxidative stress and inflammation may be seen as both causes and consequences of cellular pathophysiology in many disorders, probably in neuropsychiatric disorders such as schizophrenia, Alzheimer's disease (1).

Uric acid (UA) is a $C_5H_4N_4O_3$ (7, 9-dihydro-1H-purine-2, 6, 8(3H)-trione) heterocyclic organic compound with a molecular weight of 168 Da. Many enzymes are involved in the conversion of the two purine nucleic acids, adenine and guanine, to uric acid. Initially, adenosine mono phosphate (AMP) is converted to inosine via two different mechanisms; either first removing an amino group by deaminase to form inosine mono phosphate (IMP) followed by de-phosphorylation with nucleotidase to form inosine, or by first removing a phosphate group by nucleotidase to form adenosine followed by deamination to form inosine. Guanine mono phosphate (GMP) is converted to guanosine by nucleotidase. The nucleosides, inosine and guanosine, are further converted to purine base hypoxanthine and guanine, respectively, by purine nucleoside phosphorylase (PNP) (2).

UA is a powerful scavenger of free radicals accounting for approximately half of the antioxidant capacity of human plasma. Several studies have suggested a causal role of UA in the neurodegenerative diseases. The possible neuroprotective role of UA has been shown by its anti-oxidative action of the non-crystal form of UA because; oxidative stress plays a critical role in neurodegeneratioin including dopaminergic degeneration in Parkinson's disease. As a natural antioxidant, UA provides up to 60% of the antioxidant capacity in human blood. UA preserves the peroxidase activity of both cytosolic Superoxide Dismutase 1 (SOD and extracellular SOD_3), which defend against the formation of superoxide (O_2^-) and peroxynitrite. UA can also effectively prevent cytoskeleton from the insults caused by peroxynitrite-induced inactivation of cellular enzymes. In addition, UA is capable of binding iron and inhibits iron-dependent ascorbate oxidation, thus preventing against oxidative stress-induced injuries. As such, a reduced UA concentration may adversely lead to increased oxidative stress and damage to neural cells. However, recent studies indicate that UA's antioxidant property couldn't explain all of its beneficial effects in the CNS. One study found that astroglia are required for UA to exert its protective effects on the spinal cord against injury. UA stimulates expression of a glutamate transporter in astroglia, by which it protects neurons from glutamate-induced toxicity. Besides its capability of scavenging reactive oxygen species, UA can also indirectly confer neuronal protection via activation of astroglia. On the other hand, lower UA levels may be generated during CNS inflammation due to overconsumption of UA in scavenging excessive oxidative stress (2, 3).

Conversely, cellular studies have demonstrated that UA may also exert pro-oxidant effects depending on its chemical microenvironment. It has been suggested that the increase in UA serum levels represents an adaptive response developed by the organism against the detrimental effects of excessive oxidative stress, which characterizes cardio-metabolic and vascular diseases (4).

Studies highlighting the pathogenic mechanisms of uric acid point to an inflammatory response as the primary mechanism for inducing gout and possibly contributing to uric acid's vascular effects. Monosodium urate (MSU) crystals induce an inflammatory reaction, which are recognized by Toll-like receptors (TLRs). These TLRs then activate $NALP_3$ inflammasome. MSU also triggers neutrophil activation and further produces immune mediators, which lead to a proinflammatory response. In addition, soluble uric acid can also mediate the generation of free radicals and function as a pro-oxidant (5). It induces NADP-oxidase activation by activating redox-dependent pro-inflammatory signaling in cultured adipocytes. Additionally, UA up-regulates the expression of *Crp*, a marker of inflammation, in human vascular smooth muscle cells and endothelial cells (6). For patients with hypertension, heart failure or diabetes, hyperuricemia always predicts a high mortality. The mechanisms by which uric acid causes organ injury are still incompletely understood. However, whether it is neuroprotective or neurotoxic in the central nervous system remains controversial (7).

Adenosine is a neuromodulator of brain function that is uniquely positioned to integrate excitatory and inhibitory neurotransmission and neuroprotective actions in pathological conditions (8). Since, it 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. Its dysregulation has been implicated in pathophysiology of several neuropsychiatric disorders. In addition, adenosine inhibits the aggregation of platelets and neutrophils and can thereby reduce a localized inflammatory response (9).

Intracellular and extracellular levels of adenosine are tightly controlled by specific nucleoside transporters and several important enzymes, which include ADA and 5'-nucleotidase (5'-NT) (10). 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. In addition, overproduction of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion ($O^{\bullet-}_2$); nitric oxide ($NO^{\bullet}$) and singlet oxygen (O_2) creates a condition known as oxidative stress, resulting in the amplification of the inflammatory response (11). Recently, it has been demonstrated that the burst of xanthine oxidase mediated free radical

generation in the reperfused tissue is triggered by a large increase in substrate formation, which occurs secondary to the degradation of adenine nucleotides. Thus, increased activity of adenosine deaminase can increases the xanthine oxidase substrate formation leads to excess of free radical generation (12).

In an attempt to clarify the association of UA with the systemic inflammatory response in addition to oxidative stress, we investigated the relationship between serum UA levels and serum ADA activity as inflammatory biomarker in patients with neuropsychiatric disorders.

MATERIALS AND METHODS:

In the study total numbers of 480 subjects were included. Out of these 240 were normal healthy controls (age and sex matched controls), 240 were neuropsychiatric disorders patients distributed in four groups (including 60 patients and 60 controls in each group) as group I Schizophrenia and controls (18 years-55 years), group II- Major Depression and controls (18 years-55 years), group III- Epilepsy and controls (12 years-55 years), and group IV- Alzheimer's disease and controls (55 years-75 years). Patients were diagnosed according to DSM-IV (American Psychiatric Association Diagnostic and Statistical Manual of Mental disorders) (American Psychiatric Association 1994) (13). 30 normal healthy control volunteers of matched age and sex were recruited to participate (included in the study for comparison). All subjects provided written informed consent (from patient's relatives).

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 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 venous blood samples obtained from these subjects were used for the analysis. Serum was separated by centrifugation at 3000 g for 15 minutes and used for the estimation of MDA, UA and ADA activity respectively. The level of serum total lipid peroxide in terms of Malondialdehyde (MDA) was determined by TBARS method described by Bird (14). Serum UA level by uricase method using kit (15) and Serum ADA activity was assayed by Giusti G.L. method (16). All the reagents used were of analytical reagent grade.

Statistical analysis:

Statistical analysis between controls and patients was performed by student's 't' test using Graphpad software. The data were expressed as mean ± SD. A p value of < 0.05 was considered statistically significant. Simple correlation analysis (Pearson correlation coefficient) was used to compare continuous variables. $P<0.05$ was considered as significant.

RESULTS:

The levels of MDA, UA and ADA activity in different group patients and in controls are shown in table 1. The values of serum MDA levels in healthy controls, group I, group II, group III and group IV patients with neuropsychiatric disorders. It was observed that the levels of MDA were significantly increased in all patient groups of neuropsychiatric disorders compared to healthy controls (group I, group II and group III $p<0.0001$, group IV; $p<0.001$). Thus, results indicate that the patients with neuropsychiatric disorders are definitely subjected to oxidative stress as indicated by increased lipid peroxidation.

Table No. 1 depicts the values of serum uric acid in healthy controls and all patients of group I, group II, group III and group IV. There was significant decrease in levels of uric acid in patient groups I, II and IV (group I $p<0.001$, group II and group IV $P<0.05$) as compared to healthy controls. Group III patients shows increased levels ($p<0.05$). There was significant increase in ADA activity in all groups (group I, group III and group IV $p<0.0001$, group II $p<0.05$) as compared to healthy controls illustrate the levels of inflammation. When we analyzed the correlation between serum ADA activity and UA levels, a significant negative correlation was found between UA and ADA in all patient groups except Group III patients (Group I; $r = -0.29$, $p= 0.02$, Group II; $r = -0.28$, $p= 0.02$, Group IV; $r = -0.46$, $p= 0.0002$).

DISCUSSION:

The present study demonstrated that the serum MDA were significantly higher in all group patients compared to healthy control groups. The increased levels of MDA indicate the oxidative insults contributed by free radicals formation that attacks on lipoproteins molecules causing lipid peroxidation.

There was significant reduced levels of uric acid shown in patients with group I, II and IV (group I $p<0.001$, group II and group IV $P<0.05$) as compared to healthy controls. Group III patients shows increased levels ($p<0.05$).

Serum uric acid levels that are below normal concentrations have also been linked to a variety of disease states, including multiple sclerosis, optic neuritis, Parkinson's disease, and Alzheimer's disease. In these inflammatory diseases, a decreased UA concentration may not be able to prevent the toxicity by reactive oxygen and nitrogen species that form as a result of the inflammation (17).In these diseases, a depleted UA concentration may not be able to prevent the toxic effects caused by ROS and RNS that are form during inflammation. It is not known certainly; whether low UA levels in serum are a cause or a consequence of the diseases (5). It was found that uric acid may interfere with the invasion of inflammatory cells into the CNS and prevents development of the disease (18).

However, increased UA level has been associated with obesity, metabolic syndrome, dysglycemic conditions, diabetes mellitus, hypertension, endothelial dysfunction, cardiovascular disease, and chronic kidney disease, most likely as a consequence of westernized lifestyle and environment. However, the causal mechanisms linking elevated UA levels to cardio-metabolic and vascular disease are still unsettled (4).

Oxidative stress and inflammation are inextricably related processes. Chronic inflammation is mostly associated with levels of elevated ROS; an anti-inflammatory cascade is linked to the process of scavenging ROS concentrations (19). ADA is the enzyme for the regulation of adenosine levels (11). Therefore any change in adenosine deaminase levels will reflect to adenosine levels. Its dysregulation has been implicated in pathophysiology of several neuropsychiatric disorders. In addition, adenosine inhibits the aggregation of platelets and neutrophils and can thereby reduce a localized inflammatory response (9).

In present study there was elevated significantly in ADA activity all groups patients compared to healthy controls illustrate the levels of inflammation. Increased lipid oxidation might be due to activation of immune response followed by induction of pro-inflammatory mediators like tumor necrosis factor and interleukins or disturbance related to cellular immune system functioning (20, 21).

There is very little data available regarding ADA activity in patients with neuropsychiatric disorders. Studies by Herken et al (2007) indicated increased ADA activity in depression (22) while Dutra et al (2009) reported lower activity of ADA in schizophrenic patients (23). Our results are in accordance with observation indicated by increased activity. Interestingly, serum ADA activity is increased in medicated schizophrenia patients, but it remains to be established if this increase in related to the phenotype or to medication (24). Decreased activity of ADA suggests that the impaired immune state in depression while major depressed patients might have a greater tendency to immune dysfunction than the minor depressed ones (21). Overall, these findings support the idea that UA and ADA may negatively impact the health status or disease progress, perhaps by activating a complex vicious cycle involving inflammatory and oxidative related mechanisms.

CONCLUSION:

In conclusion we found higher serum MDA and lower UA patients than in healthy controls. Increased serum ADA activity suggesting activation of immune response followed by induction of pro-inflammatory mediators like tumor necrosis factor and interleukins or disturbance related to cellular immune system functioning. A

significant association was found between UA and ADA activity suggesting there involvement in aggravating of inflammatory processes and oxidative stress which may associate with pathophysiology of neuropsychiatric disorders. Further studies on UA and ADA, for the association of inflammation with oxidative stress in pathophysiology of 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.

Table No. 1 Indicates levels of Serum MDA (nmol/dL), UA (mg/dL) and ADA activity (U/L) in healthy control subjects and in neuropsy chiatric patients

Parameters	MDA(nmol/dL)		UA(mg/dL)		ADA(U/L)	
	Controls (n=60)	Patients (n=60)	Controls (n=60)	Patients (n=60)	Controls (n=60)	Patients (n=60)
Group I	260.82 ±26.10	352.83±45.96***	4.45±1.06	3.64±1.21**	16.16±2.67	24.85±2.53***
Group II	256.62±32.40	295.28±65.92***	4.43±1.06	3.80±1.06*	17.12±3.09	19.52±5.00*
Group III	254.98±31.93	293.01±57.08***	3.90±1.02	4.54±1.12*	17.04±3.12	24.06±2.65***
Group IV	288.70 ±38.93	318.21±54.74**	4.55±1.04	4.12±0.90*	17.97±3.26	25.10±2.45***

All values are expressed as Mean ± SD
n=Indicates the no. of subjects

***p<0.0001=Very Highly significant, **p<0.001= Highly significant, *p< 0.05= Significant,

Figure No. 1 Correlation analysis between UA and ADA in Group I patients

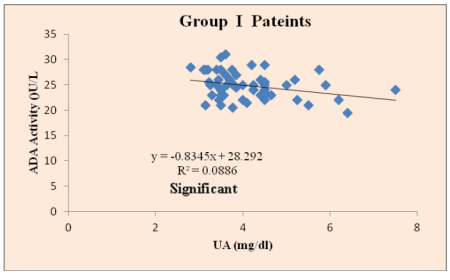


Figure No. 2 Correlation analysis between UA and ADA in Group II patients

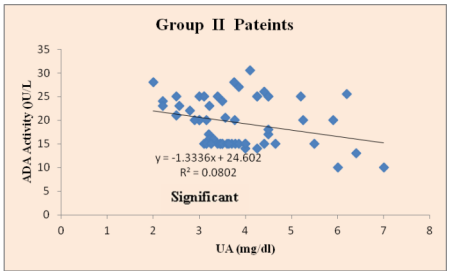


Figure No. 3 Correlation analysis between UA and ADA in Group III patients

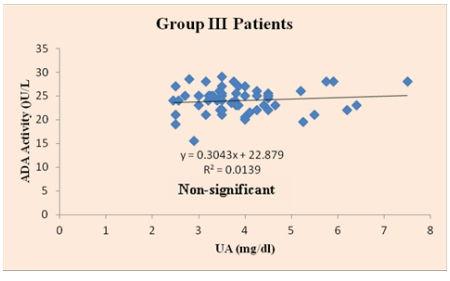
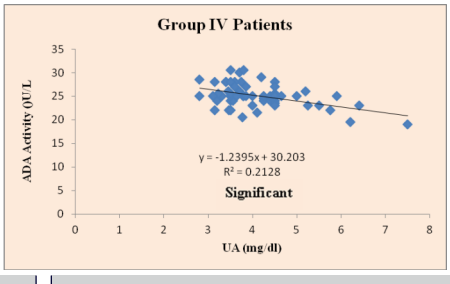


Figure No. 4 Correlation analysis between UA and ADA in Group IV patients



REFERENCES:

1. Yegin A, Ay N , Aydin O, Yargici N et al. Increased oxidant stress and inflammation in patients with chronic schizophrenia. International Journal of Clinical Medicine 2012; 3: 368-76.
2. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C,and Mollace V. Regulation of uric acid metabolism and excretion. International Journal of Cardiology 2016;213:8-14.
3. Fang P, Li X, Luo JJ Wang H, and Yang X. A Double-edged Sword: Uric Acid and Neurological Disorders Brain Disord Ther. 2013; 2 (2):109-15.
4. Spiga R, Marini MA, Mancuso E, and Fatta CD, Anastasia Fuoco, Perticone F, Andreozzi F, Mannino GC, and Sesti G. Uric acid is associated with inflammatory biomarkers and induces inflammation via activating the nf-κb signaling pathway in hepg2 cells. Arterioscler Thromb Vasc Biol. 2017; 37:1241-1249.
5. Jin M, Yang F, Yang I, Yin Y, Luo JJ, Wang H, and Yang X. Uric Acid, Hyperuricemia and Vascular Diseases. Front Biosci 2012; 17: 656-669.
6. Lu W, Xu Y, Shao X, Gao F, Li Y, Hu J, Zuo Z, Shao X, Zhou L, Zhao Y & Cen X. Uric acid produces an inflammatory response through activation of nf-κb in the hypothalamus: implications for the pathogenesis of metabolic disorders. Scientific Reports 2015; 5:12144 doi: 10.1038/srep12144.
7. Zhou Y, Fang L, Jiang L, Wen P, Cao H, et al. (2012) Uric Acid Induces Renal Inflammation via Activating Tubular NF-kB Signaling Pathway. PLoS ONE 2012;7(6):e39738.
8. Latini S and Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. Journal of Neurochemistry 2001; 79: 463-84.
9. Ramkumar V, Hallam DM and Nie Z. Adenosine, oxidative stress and cytoprotection. Jpn J Pharmacol 2001; 86:265- 74.
10. Abu Shmais GA, Al-Ayadhi LY, Al-Dbass AM and El-Ansary AK. Mechanism of nitrogen metabolism-related parameters and enzyme activities in the pathophysiology of autism. Journal of Neurodevelopmental Disorders 2012; 4:4.
11. 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.
12. Xia Y, Khatchikian G and Zweier JL. Adenosine deaminase inhibition prevents free radical-mediated injury in the postischemic heart. The Journal of Biological Chemistry 1996; 271(17): 10096-102.
13. American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Press, Washington 20.
14. Bird RP, Draper HH. Comparative study on different methods of MDA determination. Methods Enzymol; 1984; 105.
15. Caraway WT. carbohydrates in Tietz N. W. (Ed). Fundamentals of clinical chemistry. W.B. Saunder Company; 1999. pp 173-6.
16. 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.
17. Kutzing MK and Firestein BL. Altered uric acid levels and disease states. The journal of pharmacology and experimental therapeutics 2008; 324(1):1-7.
18. Hooper DC, Scott GS, Zborek A, Mikheeva T et al. Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood–CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. FASEB J 2000; 14: 691-8.
19. Yegin A, Ay N , Aydin O, Yargici N et al. Increased oxidant stress and inflammation in patients with chronic schizophrenia. International Journal of Clinical Medicine 2012; 3: 368-76.
20. Michel TM, Pulschen D and Thome J. The role of oxidative stress in depressive disorders. Current Pharmaceutical Design 2012; 18:5890-9.
21. 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.
22. 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.
23. 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.
24. 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.