INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

ALTERED ADENOSINE METABOLISM & ADENOSINE DEAMINASE ACTIVITY IN BRONCHIAL ASTHMA:-EXPLORING A NEW DIMENSION & A POSSIBLE FUTURE THERAPEUTIC TARGET.

DR.SAMIR SAHU[1], DR.BANANI JENA[2], DR. ANIRBAN PODDAR[3], DR. R. N. MANIA[4], DR. SUBHASHREE RAY[5], DR. RAKHI LUDAM[6], DR. PRITAM CHHOTRAY[7]

1. Associate Professor, Dept Of General Medicine, Ims & Sum Hospital,Bhubaneswar,Odisha.
2. Associate Professor, Dept Of Pulmonary Medicine, Ims & Sum Hospital,Bhubaneswar,Odisha
3. Pgt, Dept Of Biochemistry, Ims & Sum Hospital,Bhubaneswar,Odisha
4. Professor, Dept Of Pulmonary Medicine, Ims & Sum Hospital,Bhubaneswar,Odisha
5. Professor & Hod, Dept Of Biochemistry, Ims & Sum Hospital,Bhubaneswar,Odisha
6. Assistant Professor, Dept Of Pulmonary Medicine, Ims & Sum Hospital,Bhubaneswar,Odisha
7. Pgt, Dept Of Pulmonary Medicine, Ims & Sum Hospital,Bhubaneswar,Odisha

Accepted Date: 15/10/2015; Published Date: 27/10/2015

Abstract: Background:-Adenosine deaminase is an enzyme of purine metabolism. It catalyses the irreversible deamination of adenosine to ammonia & inosine. Adenosine is one of the main substrates of adenosine deaminase & it is well known for its bronchoconstrictor & antiarrhythmic actions. Some studies have reported a diminished adenosine deaminase activity in serum of asthmatics. **Objective:-**The objective of our study was to observe adenosine deaminase activity in serum of patients suffering from bronchial asthma & have a better understanding of this less ventured domain regarding its role in pathogenesis of bronchial asthma. **Methodology:-** Serum ADA activity was measured in total 60 asthmatic subjects & 20 healthy controls. ADA was measured by a colorimetric technique described by Giusti & Galanti. **Result:-**ADA activity in serum of asthmatics was found to be significantly decreased as compared to the healthy controls. **Conclusion:-**ADA activity in serum of asthmatics possibly decreases leading to accumulation of adenosine & its subsequent deleterious effects such as bronchoconstriction. This understanding might help us to devise tools to enhance ADA activity in asthma. Thus we may be looking at a lucrative therapeutic target in the near future in the form of ADA.

Keywords: Adenosine Deaminase, Asthma, Diagnostic Marker, 5'nucleotidase.

Corresponding Author: DR. ANIRBAN PODDAR

Access Online On:

www.ijprbs.com

How to Cite This Article:

Anirban Poddar, IJPRBS, 2015; Volume 4(5): 328-335

PAPER-QR CODE

INTRODUCTION

Bronchial asthma is one of the most common chronic illnesses worldwide with an estimated 300 million people already affected by it[1].The study conducted by Jindal etal in 2007 reports that 2.38% of the total Indian population suffers from asthma[2].Inflammation of the airways is a cardinal feature of asthma along with hyper responsiveness of the airways leading to excessive bronchoconstrictor response to multiple inhaled triggers[3].It has been proved beyond doubt that a specific pattern of airway inflammation is related with airway hyper responsiveness which is further correlated with variable airflow obstruction[4].The involvement of an inflammatory cascade in pathogenesis of asthma has been reported by many researchers, the participants of which are various inflammatory mediators such as interleukins, histamine, adenosine, prostaglandins secreted by macrophages, eosinophils, mast cells & lymphocytes[4].

Adenosine is a purine nucleoside that has been found to play an important role in many biological processes such as energy generation & protein metabolism, but in the past decade many researchers have reported its involvement in the pathogenesis of many inflammatory disorders including bronchial asthma[5].Inflammatory tissue damage & local tissue hypoxia in the inflamed areas account for one of the prime causes of adenosine accumulation, which is released from immune & nonimmune cells [6,7].Rapid metabolism of ATP due to increased energy demands in certain cells such as platlets, mast cells & endothelial cells also contributes to release & accumulation of adenosine[8].Adenosine thus accumulated interacts with specific G protein coupled adenosine receptors on inflammatory & immune cells to regulate their functions[9].Role of adenosine in bronchial asthma was unmasked almost 20 years ago when a group of asthma patients exhibited bronchoconstriction in response to aerosolised adenosine while normal individuals didn't display a response[10].Adenosine acts through A_{2B} & A_3 receptors & causes mast cell activation & degranulation leading to the release of inflammatory & nociceptive mediators like cytokines which account for the bronchoconstriction & the airway hyperresponsiveness[11-14].Adenosine also causes liberation of inflammatory cytokines from smooth muscles via A_2B receptors[15.]

The bioavailability of adenosine plays an important factor in asthma. Adenosine occupies an important position in purine metabolism. It primarily has three fates. It might either be phosphorylated to the nucleotide level, might undergo deamination to inosine or undergo dephosphorylation to the base level. But adenosine dephosphorylase has been found only in the bacterial system[16, 17]. Phosphorylation is favoured at low concentrations whereas at high concentration deamination predominates[18]. Under normal conditions adenosine is derived from intracellular adenosine monophosphate (AMP) that is present at low levels in the cell, mostly derived from the catabolism of high energy adenosine phosphates (ATP, ADP)[19]. Intra-cellular (AMP) is formed and shortly reconverted to ADP and ATP as part of the energy cycle.

However, under conditions of high-energy demand, AMP cannot be reconverted and it is metabolised to adenosine by 5′-nucleotidase (plasma membrane bound mainly as well as cytoplasmic)[20]. Intracellular levels of adenosine are kept low by its conversion to AMP by the enzyme adenosine kinase and to inosine by adenosine deaminase, but when energy demands are greater as in inflammation, deamination predominates[21]. Extracellular adenosine diffuses back into the cell through the operation of an energy-independent nucleoside transporter[22]. 5′-nucleotidase (5′-NT, E.C. 3.1.3.5) catalyzes the hydrolysis of the phosphoric ester bond of 5′-ribonucleotides to the corresponding ribonucleoside and phosphate. The main function of 5′-NT is the hydrolysis of AMP to adenosine. Adenosine Deaminase (ADA,EC3.5.4.4),a key enzyme in the purine salvage pathway, catalyzes the irreversible hydrolytic deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. Two different isoenzymes of ADA designated as ADA1 and

ADA2 were found in mammals and lower vertebrates[23].

The production, release & metabolism of adenosine depend on the relevant enzyme activities influencing its metabolism.5'nucleotidase & adenosine deaminase are the two key enzymes regulating adenosine metabolism. Studies have reported in the past about enhanced 5'nucleotidase activity in asthmatics[5].We hypothesised that a decreased ADA activity or an increased 5'nucleotidase activity or both might contribute to the pathogenesis of asthma. Since very few studies have been conducted that elicit ADA activity in asthmatics, so we proposed to carry out the present study to determine whether any correlation exists between ADA activity and the pathogenesis of asthma.

METHODOLOGY:-

The subjects for the study were divided into four groups:-

1. **Group A**:-Group A comprised of 20 newly diagnosed cases of bronchial asthma,who had presented to the pulmonary medicine OPD at IMS & SUM HOSPITAL.They were classified as "mild persistent" cases of asthma.

2. **Group B**:-Group B comprised of 20 established cases of bronchial asthma who were classified as "moderately persistant" cases of asthma.

3. **Group C**:-This group comprised of 20 established cases of "severely persistent"asthma.

4. **Group D**:- This group comprised of 20 age & sex matched healthy controls with no history of major illness or hospitalisation & who presented to the general medicine opd for general health checkup.

The diagnosis & classification of subjects as "mild persistent"," moderate persistent" & "severe persistent" was based on the "NATONAL ASTHMA EDUCATION & PREVENTION PROGRAMME'S" "EXPERT PANEL REPORT-3 (EPR 3)" guidelines (2007).

A written informed consent was taken from all the subjects as a proof of their willingness to be a part of this study. The study was presented before the institutional ethical committee which follows the Helsinki guidelines of human research & an ethical clearance was obtained. The authors hereby declare that no conflict of interest exists whatsoever.

ADA ESTIMATION

5 ml of venous blood was obtained from each subject with full aseptic precautions for estimation of serum ADA .ADA was estimated by a commercially available kit supplied by Tulip Diagnostics, Goa using auto analyser PHOTOMETER 5010.The kit employs the colorimetric technique of ADA estimation as illustrated by Giusti & Galanti[24] in 1984.ADA exists as two isoenzymes in humans namely ADA 1 & ADA 2[25].ADA 1 is present in all tissues of the body whereas ADA 2 is present mainly in serum originated from the macrophage-monocyte system[25].

The initial reading of each sample was taken & this gave us the total ADA activity (t-ADA) in the serum. EHNA (erythro 9, 2 hydroxy,3nonyl adenine) which is a selective inhibitor of ADA 1 was procured from SIGMA ALDRICH. 1µg of EHNA was added to each sample & a repeat reading was taken. This gave us the exclusive ADA 2 activity. Subtracting ADA 2 from t-ADA gave us ADA 1 activity.

RESULTS & DISCUSSION:-

Our study revealed that ADA activity was grossly diminished in asthmatic subjects as compared to the healthy controls. The degree of diminition although showed a significant degree of variation among the three asthmatic groups in the study.

In asthma, the total activity of ADA, ADA1 and ADA2 in serum decreased as compared to healthy controls. The difference was significant between control and moderate persistent asthmatics [($p < 0.0001$) for total ADA and ADA1, ($p < 0.001$) for ADA2], control and severe persistent asthmatics [($p < 0.0001$) for total ADA, ADA1 and ADA2]. However, the difference was not significant between mild persistent and healthy controls. Further, the decrease in total ADA, ADA1 and ADA2 was significant between mild and moderate persistent asthmatics and between mild and severe persistent asthmatics ($p<0.05$).

These findings of ours are similar to what J. Sharma etal[5] have reported. Several other studies in the recent past have reported altered ADA activity to be associated with pathogenesis of other diseases such as diabetes mellitus[26,27]. Thus it is implied that this enigmatic moonlighting

enzyme plays a pivotal role in metabolic processes in the body & surely holds the key that will unlock newer dimensions of treatment for various diseases which will not only modify the disease process but will cure the disease from within.

ADA & ITS ISOFORMS	GROUP A	GROUP B	GROUP C	GROUP D
t-ADA	10.69±0.32	8.29±0.31	7.71±0.33	11.16±0.39
ADA 1	3.53±0.23	2.24±0.16	2.43±0.18	3.77±0.20
ADA 2	7.16±0.19	6.06±0.24	5.28±0.23	7.39±0.29

Table:-Total ADA & its isoform's activity expressed as mean±SD in the three asthmatic groups & the control group respectively.

CONCLUSION & FUTURE SCOPE:-

Our study has revealed that ADA activity is significantly reduced in asthmatics as compared to healthy controls. Other studies conducted previously on 5'nucleotidase, another key player in adenosine metabolism by various researchers have revealed that its activity is significantly enhanced in asthma[5].Thus it may be inferred that enhanced 5'nucleotidase activity leads to excessive production of adenosine by degradation of nucleotides. Simultaneously a reduced ADA activity leads to decreased catabolism of adenosine. The final resultant of this interplay of enzymes leads to accumulation of adenosine & this explains the consequent bronchoconstriction mediated by the A_{2B} & A_3 adenosine receptors in lung. This understanding of adenosine metabolism & interplay of enzymes involved in adenosine metabolism & its correlation in asthma might help us devise newer potential tools to cure asthma in future.

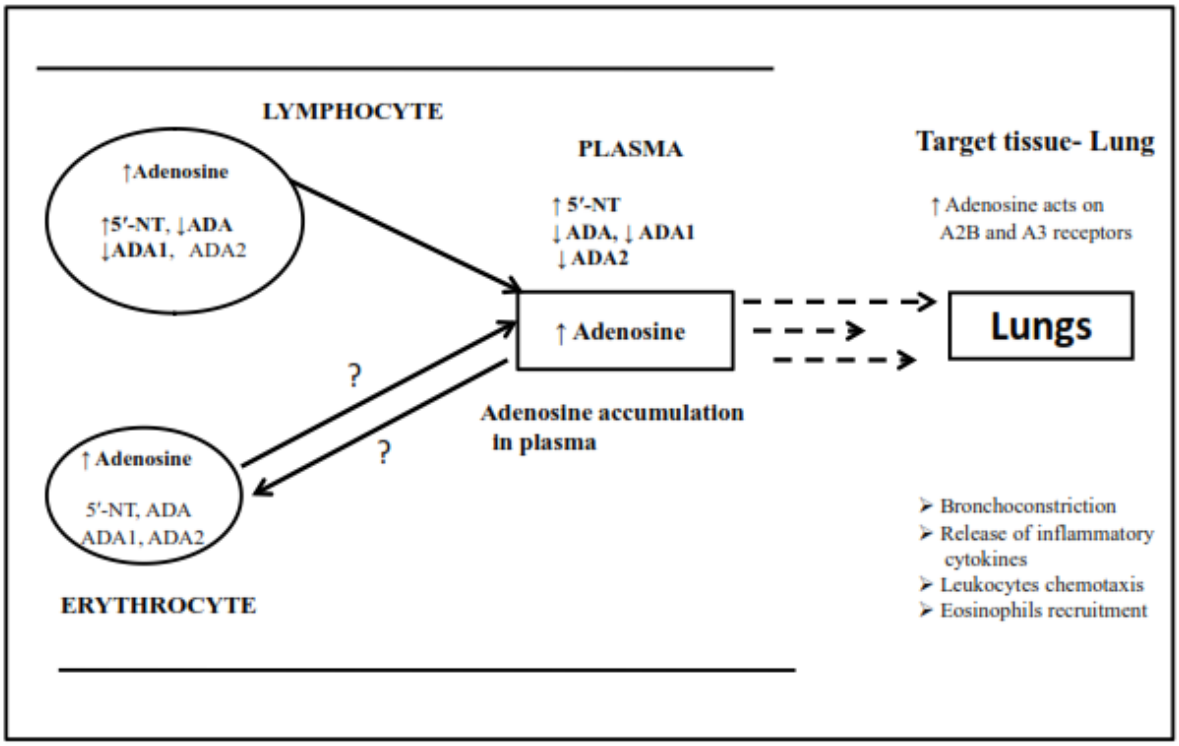

Fig:-Adenosine metabolism in blood of patients from asthma & a correlation of the microscopic events with macroscopic symptoms.(Adapted from Sharma J.P etal (2015) Changes in adenosine metabolism in asthma: A study on adenosine,5'NT,ADA & its isoenzyme levels in serum, erythrocytes & lymphocytes.*OJRD,*5,33-49.)[5]

REFERENCES:-

1. Asher, M.I., Montefort, S., Björkstén, B., Lai, C.K., Strachan, D.P., Weiland, S.K. and Williams, H. (2006) ISAAC Phase Three Study Group. Worldwide Time Trends in the Prevalence of Symptoms of Asthma, Allergic Rhinoconjunctivitis, and Eczema in Childhood: ISAAC Phases One and Three Repeat Multicountry Cross-Sectional Surveys. Lancet, 368, 733-743.

2. Jindal, S.K. (2007) Bronchial Asthma the Indian Scene. Current Opinion in Pulmonary Medicine, 13, 8-12.

3. McFadden Jr., E.R., Kasper, D.L., Braunwald, E., Fauci, A.S., Longo, D.L., Hauser, S.L. and Jameson, J.L. (2005) Harrison's Principles of Internal Medicine. 16th Edition, McGraw Hill, New York, 1508-1516.

4. Busse, W.W. and Lemanske, R.F. (2001) Asthma. New England Journal of Medicine, 344, 350-362.

5. Sharma J.P etal (2015) Changes in adenosine metabolism in asthma: A study on adenosine, 5'NT, ADA & its isoenzyme levels in serum, erythrocytes & lymphocytes. OJRD, 5, 33-49.

6. Winn, H.R., Rubio, R. and Berne, R.M. (1981) Brain Adenosine Concentrations during Hypoxia in Rats. American Journal of Physiology, 241, 235-242.
7. Van, B.H., Goossens, F. and Wynants, J. (1987) Formation and Release of Purine Catabolites during Hypoperfusion,Anoxia, and Ischemia. American Journal of Pathology, 252, 886-893.
8. Linden, J. (2001) Molecular Approach to Adenosine Receptors: Receptor-Mediated Mechanisms of Tissue Protection.Annual Review of Pharmacology and Toxicology, 41, 775-787.
9. Newby, A.C. (1984) Adenosine and the Concept of Retaliatory Metabolites. Trends in Biochemical Sciences, 9, 42-44.
10. Cushley, M.J., Tattersfield, A.E. and Holgate, S.T. (1983) Inhaled Adenosine and Guanosine on Airway Resistance in Normal and Asthmatic Subjects. British Journal of Clinical Pharmacology, 15, 161-165.
11. Church, M.K., Hughes, P.J. and Vardey, C.J. (1986) Studies on the Receptor Mediating Cyclic AMP-Independent Enhancement by Adenosine of IgE Dependent Mediator Release from Rat Mast Cells. British Journal of Pharmacology,87, 233-242.
12. Salvatore, C.A., Jacobson, M.A., Taylor, H.E., Linden, J. and Johnson, R.G. (1993) Molecular Cloning and Characterization of the Human A Adenosine Receptor. Proceedings of the National Academy of Sciences of the United States of America, 90, 10365-10369.
13. Tigani, B., Hannon, J.P., Mazzoni, L. and Fozard, J.R. (2000) Effects of Wortmannin on Bronchoconstrictor Responses to Adenosine in Actively Sensitised Brown Norway Rats. European Journal of Pharmacology, 406, 469-476.
14. Tilley, S.L., Wagoner, V.A., Salvatore, C.A., Jacobson, M.A. and Koller, B.H. (2000) Adenosine and Inosine Increase Cutaneous Vasopermeability by Activating A Receptors on Mast Cells. Journal of Clinical Investigation, 105, 361-367.
15. Young, H.W., Molina, J.G., Dimina, D., Zhong, H., Jacobson, M., Chan, L.N., Chan, T.S., Lee, J.J. and Blackburn, M.R. (2004) A Adenosine Receptor Signalling Contributes to Airway Inflammation and Mucus Production in Adenosine Deaminase-Deficient Mice. The Journal of Immunology, 173, 1380-1389.
16. Thompson, L.F. and Seegmiller, J.E. (1980) Adenosine Deaminase Deficiency and Severe Combined Immunodeficiency Disease. Advances in Enzymology and Related Areas of Molecular Biology, 51, 167-210.
17. Cirulea, F., Saura, C., Canela, E.I., Mallot, J., Lluis, C. and Franco, R. (1996) Adenosine Deaminase Affects Ligand Induced Signalling by Interacting with Cell-Surface Adenosine Receptors. FEBS Letters, 380, 219-223.
18. Herrara, C., Casado, V., Cirulea, F., Schofield, P., Mallol, J., Lluis, C. and Franco, R. (2001) Adenosine A Receptors Behave as an Alternative Anchoring Protein for Cell Surface Adenosine Deaminase in Lymphocytes and Cultured Cells. Molecular Pharmacology, 59, 127-134.

19. Fredholm, B.B., Ijzerman, A.P., Jacobson, K.A., Klotz, K.N. and Linden, J. (2001) International Union of Pharmacology XXV: Nomenclature and Classification of Adenosine Receptors. Pharmacological Reviews, 53, 352-527.
20. Dunwiddie, T.V., Diao, L. and Proctor, W.R. (1997) Adenine Nucleotides Undergo Rapid, Quantitative Conversion to Adenosine in the Extracellular Space in Rat Hippocampus. The Journal of Neuroscience, 17, 7673-7682.
21. Polosa, R. (2002) Adenosine-Receptor Subtypes: Their Relevance to Adenosine Mediated Responses in Asthma and Chronic Obstructive Pulmonary Disease. European Respiratory Journal, 20, 488-496.
22. Andrew, J.H., Jaclyn, R., Stonebraker, C.A., Van, H., Eduardo, L., Richard, C.B. and Maryse, P. (2007) Adenosine Deaminase 1 and Concentrative Nucleoside Transporters 2 and 3 Regulate Adenosine on the Apical Surface of Human Airway Epithelia: Implications for Inflammatory Lung Diseases. Biochemistry, 46, 10373-10383.
23. Ratech, H., Thorbecke, G.J., Merdith, G. and Hirschhorn, R. (1981) Comparison and Possible Homology of Isoenzymes of Adenosine Deaminase in Aves and Humans. Enzyme, 26, 74-84.
24. G. Giusti and B. Galanti, “Colorimetric method. Adenosine deaminase,” in Methods of Enzymatic Analysis, H. U. Bergmeyer, Ed., pp. 315–323, Verlag Chemie, Weinheim, Germany, 3rd edition, 1984.
25. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role.Eur Respir J 1996; 9: 632-3.
26. Poddar, A etal(2015) Serum Adenosine deaminase activity:-A promising glycemic marker in uncomplicated type 2 diabetes mellitus. IJPRBS, Volume 4(2):71-79.
27. Poddar, A etal (2015) Serum adenosine deaminase activity:-A possible explanation for hyperactive immune status & onset of nephropathy in diabetic subjects. IJPRBS, Volume 4(3):211-219.