International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Comparative Study of Multiple Sclerosis Therapy by Interferon-β and Methylprednisolone of Iraqi Patients via Adenosine Deaminase Activity

Hazima Mossa K. Alabassi[1], Zahraa Hussein M. Kadri[2], Hind Qusay Ismail[3], Yasamine Jumaa Kadem[4], Hadeel Khlil Rahman[5]

Department of Biology, College of Education Ibn Al-Haitham, University of Baghdad.

Abstract: ***A total of 60 patients with multiple sclerosis of both gender (age range: 25-60 years) treated with Interferon-β and Methylprednisolone in addition to 60 individual divided into two groups 30 as untreated (early onset) patients, all of them were under remission state and 30 individual considered as control were studied to investigate specific activity of adenosine deaminase in serum samples. The results administrated a significantly decreasing of specific activity of adenosine deaminase in all groups which were 139.94±11.71, 146.91±15.52 and 160.26±16.87 U/mg protein respectively as compared with control 195.88±16.61 U/mg protein. The study aimed to revelation which of drugs (Interferon-β or Methylprednisolone) has a stronger effect in patient treatment as well as adenosine deaminase could suggest to be a biomarker for early detection of Multiple Sclerosis patients and Methylprednisolone consideration a therapeutic drug better than Interferon-β.***

Keywords: Multiple sclerosis, Methylprednisolone, Interferon Beta (IFN-β), Adenosine deaminase enzyme

1. Introduction

Multiple sclerosis is a chronic inflammatory demyelinating disorder of the central nervous system with various degrees of axonal damage (1). It is characterized by inflammation and damage to the insulation shield that surrounds nerve fibers (myelin), resulting in progressive neurological injury, and a number of debilitating symptoms (2). Etiology of MS is probably multifactorial, related to genetic, environmental, and several other factors (3). The sensitization and first activation of autoreactive CD4+T lymphocytes occurs in the peripheral blood. Through series of interactions they pass the blood brain barrier and invade the central nervous system. After second activation and in connection with other immune cells autoreactive T cells develop pathological process, leading to focal immune demyelination and axonal loss in the brain and spinal cord(4). This lesion lead both innate and adaptive immune cells infiltrate into the central nervous system by crossing the blood brain barrier and thereafter release a variety of pro-inflammatory cytokines (5). One of many cytokines recently found their relating with multiple sclerosis is IL-3. It expression by mononuclear cells and found to be either down regulated or up regulated in multiple sclerosis patients compared with controls(6).

The ADA is an enzyme (EC 3.5.4.4) that is involved in the metabolism of purine and catalyzes the hydrolytic deamination of adenosine to inosine, and deoxyadenosine to deoxyinosine. It has a crucial role in proliferation and differentiation of lymphoid cells, especially T lymphocytes and maturation of monocytes to macrophages (7,8). Adenosine deaminase plays an important physiological role in regulation of the effects of these metabolites on immunological, neurological and vascular systems (9). Adenosine is produced by all cells and signals through adenosine receptors, which are widely distributed in all tissues (10). It affects multiple tissue and organ functions including pancreas, liver, kidneys, skeletal muscle, heart, vascular tissue etc.(11,12). Several cell types such as lymphocytes, mast cells, macrophages, eosinophils, neutrophils and epithelial cells produce inflammatory changes by release of various mediators like adenosine, histamine, kinin, leukotrienes, prostaglandins, platelet-activating factor, chemokines and cytokines (13,14). INF-β treatment reduces the relapse rate in multiple sclerosis (MS) but the exact mechanism has remained elusive, CD73 (ecto-5-nucleotidase) is an ectoenzyme, which produce adenosine from adenosine monophosphate (AMP) precursor by enzymatic dephosphorylation. AMP is present at site of inflammation, and more importantly adenosine. The product of CD73, is possess both anti-inflammatory and neuroprotective activity. They observed that IFN-β increases the expression of ecto-5 nucleotidase on endothelial cells both in vitro and in vivo so treated patient with INF-β will increase the soluble serum CD73 so adenosine will increase and an anti-inflammatory effect in CNS will occur (15). Also Sotnikov and Louis (16) revealed that ecto-5 nucleotidase, CD73, catalyzes the phosphohydrolysis of ATP to adenosine which regulate downstream inflammatory signaling. In vitro IFN-β reduces the production of Th17 cells which have a role in pathophsiology of MS (17). Some people with multiple sclerosis develop antibodies (neutralising Ab) to the INF-β drugs that may reduce it effect (18). Methylprednisolone is a corticosteroid with anti-inflammatory action. It is used to treat acute exacerbations in patients with MS. The reduction of the inflammatory cycle is related to several type of action which included damping the inflammatory cytokine cascade, inhibit the activation of T cells, decrease the extravasation of immune cells to CNS, and indirectly decrease the cytotoxic eccect of nitric oxide and tumor necrosis factor-α (19).

In the central nervous system adenosine is a potent neuromodulator that regulated sleep, arousal, neuronal excitability, cerebral blood flow and inflammation (20). Under pathological events or increased metabolic demand, adenosine triphosphate (ATP) metabolism and adenosine levels increase (21). Adenosine catabolism is controlled by

two enzymes: adenosine deaminase converts adenosine to inosine, which is typically negligible in the central nervous system, although this may change under pathological conditions (22). Adenosine kinase converts adenosine to adenosine monophosphate following its uptake into the cell via nucleoside transporters (23). Accordingly, the present study was designed to assess the specific activity of ADA in serum of multiple sclerosis of Iraqi patients whose undergo to two types of drugs Methylprednisolone(MP) and Interferon Beta (IFN-β), and to determine its usefulness as a diagnostic biomarker.

2. Materials and Methods

Patients and Controls: A total of sixty adult individuals of both gender (age range: 25-60 years) attending the consulting clinic of Baghdad Teaching Hospital at Medicine City during the period March- May 2016, were enrolled in the study. They were clinically examined by the consultant medical staff, and based on a clinical examination, magnetic resonance image and immunological tests; the subjects were distributed into three groups; 30 untreated as early onset patients, 30 treated with methylprednisolon (MP) and 30 treated with Interferon Beta (IFN-β) as well as 30 healthy individuals represent controls. From each participant, 3 ml of venous blood were collected. The blood was left for 15 minutes to clot at room temperature, and then it was centrifuged (2000 rpm for 15 minutes) and the separated serum was frozen at -20°C until assessment. This assay has been done in Service Laboratory in College of Education For Pure Sciences Ibn-Al Haitham.

Adenosine deaminase specific activity in serum: The total activity of ADA in serum was first determined, and after protein estimation (24), the specific activity of ADA was obtained and expressed as Unit/mg protein (25). The method is based on the enzymatic deamination of adenosine to inosine, which is converted to hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase. Hydrogen peroxide is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline and 4-aminoantipyrine in the presence of peroxidase to generate quinone dye which is monitored in a kinetic manner.

Statistical analysis: Data are presented as mean ± standard error (S.E.), and differences between means were assessed by the Student t test. The analyses were carried out using the statistical package SPSS version 13.

3. Results and Discussion

The mean of adenosine deaminase (ADA) specific activity was significantly decreased in Early onset (Non treat) patients (139.94±11.71 U/mg protein) as compared with controls (195.88±16.61 U/mg protein). Also the results discovered that the patients treated with both drugs (INF-β and Methylprednisolone) significantly increasing of adenosine deaminase (ADA) specific activity as compared with Early onset patients (negative control) (146.91±15.52 and 160.26±16.87 U/mg protein, respectively).While they showed results less than controls. As well the treating with Methyprednisolone showed a better results of adenosine deaminase (ADA) specific activity than treating with IFN-β this could be proved that the Methyprednisolone is the prefer therapy drug for Multiple Sclerosis patients than IFN-β (Table 1).

Table 1: Specific activity of adenosine deaminase in serum of multiple sclerosis patients

Groups	No.	Specific Activity of ADA (Unit/mg protein)		
		Mean ± S.E.	Minimum	Maximum
Controls	30	195.88±16.61^{B}	76.13	306.74
Early (Non treat)	30	139.94±11.71^{A}	79.13	209.46
IFN-β treat	30	146.91±15.52^{A}	35.61	210.13
Methylprednisolone treat	30	160.26±16.87^{AB}	88.50	313.76

*Different letters represent significant difference ($P \leq 0.05$) between means of columns (Duncan test).

Adenosine deaminase is an important enzyme converts adenosine and 2-deoxyadenosine to inosine and 2-deoxyinosine, respectively and amonia (26). Inosine is formed both intracellularly and extracellularly (27).The deamination of adenosine to inosine occurs mainly at high intracellular concentrations of adenosine, which are associated with many forms of cellular stress (28).Although both adenosine and inosine are present constitutively at low levels in the extracellular space, metabolically stressful conditions, such as those that occur during injury, ischemia and inflammation, dramatically increase their extracellular concentrations (29). Moreover many studies confirmed the adenosine has a neuromodulator effects in the central nervous system, playing a crucial role in neuronal excitability and synaptic/nonsynaptic transmission in the hippocampus and basal ganglia (30,31). Also adenosine is involved in cellular energy and purine metabolism, but it is also released to or produced in the extracellular medium where it binds to the cell membrane adenosine receptors and its action is determined by the receptor to which it binds (32). Furthermore it was recognized that inosine can also bind to adenosine receptors and initiate intracellular signaling events (33).

Our results showed a significant decreasing in adenosine deaminase specific activity in serum of early onset group explanation for this decreasing may contribute to conformational change in adenosine receptors. So these results compatible with many studies reported that the changes in normal levels of enzyme in a wide variety of pathologies (34). Among there pathologies is neurological disorder such as multiple sclerosis (35), meningitis (36), fibromyalgia (37), depression (38) and panic disorder (39).

In addition the determination of adenosine deaminase specific activity of two types of drugs suggested that Methylprednisolone(MP) indicated to be better, giving us an idea for different response to treatments and preferable as therapy may be for possessing immunosuppressive or immunomodulatory properties than Interferon Beta (IFN-β). According to the present study we can conclude that adenosine deaminase is a good tool for detection and follow the progression of Multiple Sclerosis and adenosine is consider a new therapeutic goal to treat this disease.

References

[1] Haines J.D., Inglese M. and Casaccia P. (2011). Axonal damage in multiple sclerosis. Mt Sinai J Med., 78: 231-243.
[2] Oliveira M.S.M., Pires J. and Saudade R.J.(2015). Association of autoimmune hepatitis and multiple sclerosis: a coincidence?. Galicia Clin ,76:124-126.
[3] Mandia D., Ferraro O.E., Nosari G. , Montomoli C., Zardini E. and Bergamaschi R.(2014). Environmental Factors and Multiple Sclerosis Severity: A Descriptive Study. Int. J. Environ. Res. Public Health,11:6417-6432.
[4] Garg N. and Smith T.W. (2015). An update on immunopathogenesis, diagnosis, and treatment of multiple sclerosis. Brain Behav 5: 2-13.
[5] Kawakami N. (2016). In vivo imaging in autoimmune diseases in the central nervous system.Allergology International 65 : 235-242.
[6] Kerstin Renner K., Hellerbrand S., Hermann F., Riedhammer C., Talke Y., Schiechl G., Gomez M.R., Kutzi S., Halbritter D., Goebel N., Brühl H., Weissert R. and Mack M.(2017). IL-3 promotes the development of experimental autoimmune encephalitis. insight.jci.org., 16:1-13.
[7] Pimentela V.C., Pinheiroa F.V., De Bonaa K.S., Maldonadob P.A., da Silvab C.R., de Oliveirab S.M., Ferreirab J., Bertonchelic C.M., Schetingerb M.R., Da Luzc S.C.A. and Moretto M.B.(2011). Hypoxic–ischemic brain injury stimulates inflammatory response and enzymatic activities in the hippocampus of neonatal rats. Brain Research, 1388:134-140.
[8] Salmanzadeh S., Tavakkol H., Bavieh K. and Alavi S.M. Compston, A. (2015). Diagnostic Value of Serum Adenosine Deaminase (ADA) Level for Pulmonary Tuberculosis. Jundishapur J Microbiol., 8: 1-5.
[9] Whitmore K.V. and Gaspar H.B.(2016). Adenosine deaminase deficiency more than just an immunodeficiency. Frontiers in Immunology, 7:1-13.
[10] Oskouie F.G., Shameli A., Yang A., Desrosiers M.D., Mucsi A.D., Blackburn M.R., Yang Y., Santamaria P. and Shi Y.(2011). High Levels of Adenosine Deaminase on Dendritic Cells Promote Autoreactive T Cell Activation and Diabetes in Nonobese Diabetic Mice. J Immunol.,186:6798-6806
[11] Poddar A., DAS S., Singh B., Sahoo A.K., A. K. Rana A.K., Behera D.D. (2015). Serum adenosine deaminase activity. A promising glycemic marker in uncomplicated type 2 diabetes mellitus. International Journal of Pharmaceutical research and Bio-Science., 4:71-79.
[12] Khemka V.K., Bagchi D., Ghosh A., Sen O., Bir A., Chakrabarti S., and Anindita B.(2013). Raised Serum Adenosine Deaminase Level in Nonobese Type 2 Diabetes Mellitus. The Scientific World Journal, 2013:1-5.
[13] Busse, W.W. and Lemanske, R.F. (2001) Asthma. New England Journal of Medicine, 344: 350-362.
[14] Sharma J., Menon B.K., Vijayan V.K. and Bansal S.K.(2015). Changes in Adenosine Metabolism in Asthma. A Study on Adenosine, 5′-NT, Adenosine Deaminase and Its Isoenzyme Levels in Serum, Lymphocytes and Erythrocytes. Open Journal of Respiratory Diseases, 5:33-49.
[15] Airas L., Niemelä J., Yegutkin G. and Jalkanen S.(2007). Mechanism of action of IFN-beta in the treatment of multiple sclerosis: a special reference to CD73 and adenosine. Ann N Y Acad Sci.,1110: 641-648.
[16] Sotnikov I and Louis N.A.(2010). CD73-Dependent Regulation of Interferon AlphaA and Interleukin-10 in the Inflamed Mucosa. The Scientific World Journal,10:2167-2180.
[17] Mitsdoerffer M. and Kuchroo V.(2009). New pieces in the puzzle: how does interferon-beta really work in multiple sclerosis?.Ann Neurol., 65:487-498.
[18] Creeke P.I.and Farrell R.A.(2013). Clinical testing for neutralizing antibodies to interferon-β in multiple sclerosis. Therapeutic Advances in Neurological Disorders, 1:3-17.
[19] İşeri P., Başyiğit I., Özerdem Ç., Başyiğit H., Efendi H. and Yıldız F.(2013). The Effect of Pulse Methylprednisolone Plus Theophylline Treatment on Clinical, Pulmonary and Inflammatory Markers in Relapses of Multiple Sclerosis.Balkan Med J., 30:33-36.
[20] Smith M.D., Bhatt D.P., Geiger J.D. and Rosenberger T.A.(2014). Acetate supplementation modulates brain adenosine metabolizing enzymes and adenosine A_{2A} receptor levels in rats subjected to neuroinflammation. Journal of Neuroinflammation,11: 99.
[21] Shepel P.N., Ramonet D., Stevens P. and Geiger J.D.(2005). Purine level regulation during energy depletion associated with graded excitatory stimulation in brain. Neurol.Res., 27:139-148.
[22] Dunwiddie T.V. and Masino S.A. (2001). The role and regulation of adenosine in the central nervous system. Annu.Rev.Neurosci.,24:31-55.
[23] Boison D.(2006). Adenosine kinase, epilepsy and stroke: mechanisms and therapies.
[24] Trends Pharmacol Sci., 27:652-658.
[25] Bradford,M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantitation of protein utilizing, the principle of protein dye binding Aral. Biochemistry, 72: 248-254.
[26] Giusti,G. (1981). Adenosine deaminase. In: Bergmeyer, H.U.(Ed.) Methods of Enzymatic Analysis. 2nded. Academic Press., 2: 1092-1099.
[27] Cortes A., Gracia E., Moreno E., Mallol J., Lluis C., Canela E.I. and Casado V.(2015). Moonlighting adenosine deaminase: A target protein for drug development, Medical Research Review; (1): 85-125.
[28] Hasko G., Sitkovsky M.V. and Szabo C.(2004).Immunomodulatory and neuroprotective effects of inosine. TRENDS in Pharmacological Sciences,3:152-157.
[29] Deussen A. (2000). Metabolic flux rates of adenosine in the heart. Naunyn Schmiedebergs Arch. Pharmacol., 362: 351-363.
[30] Sachdeva S. and Gupta M.(2013). Adenosine and its receptors as therapeutic targets: An overview. Saudi Pharmaceutical Journal., 3: 245-253.
[31] Boison D. (2008). Adenosine as a neuromodulator in neurological diseases. Curr Opin Pharmacol., 8: 2-7.
[32] Sperlagh B. and Vizi E.S.(2011). The role of extracellular adenosine in chemical neurotransmission in the hippocampus and basal ganglia: Pharmacological

and clinical aspects. Curr.Top. Med. Chem., 11:1034-1046.
[33] Fredholm B.B., Johansson S., Wang Y.W.(2011). Adenosine and the regulation of metabolism and body temperature. Adv. Pharmacol., 61:77-94.
[34] Gomez G. and Sitkovsky M.V. (2003). Differential requirement for A2a and A3 adenosine receptors for the protective effect of inosine in vivo.Blood 102:4472-4478.
[35] Cristalli G, Costanzi S, Lambertucci C, Lupidi G, Vittori S, Volpini R and Camaioni E. (2001) Adenosine deaminase: Functional implications and different classes of inhibitors. Med Res Rev,21: 105- 128.
[36] Spanevello R.M., Mazzanti C.M., Schmartz R., Thome G., Bagatini M., Correa M., Rosa C., Stefanello N., Belle L.P., Moretto M.B., Oliveira L., Morsch V.M. and Schetinger M.R.(2010).The activity and expression of NTPDase is altered in lymphocytes of multiple sclerosis patients. Clin.Chim.Acta., 411: 210-214.
[37] Belagavi A.C. and Shalini M. (2011). Cerebrospinal fluid C reactive protein and adenosine deaminase in meningitis in adults. J Assoc Physicians India, 59: 557-560.
[38] Guieu R. ,Guedj E. ,Giorgi R., Dousset A. ,Tuzzolino V. ,By Y. ,Leveque J.M. ,Peragut J.C. ,Regis J. ,Ruf J., Fenouillet E. and Roussel P. (2012). High cell surface CD26-associated activities and low plasma adenosine concentration in fibromyalgia. Ann Rheum Dis ,71:1427-1428.
[39] Herken H., Gurel A., Selek S., Armutcu F., Ozen M.E., Bulut M., Kap O., Yumru M., Savas H.A. and Akyol O.(2007). Adenosine deaminase, nitric oxide, superoxide dismutase and xanthine oxidase in patients with major depression: Impact of antidepressant treatment. Arch Med Res, 38:247-252.
[40] Ozdemir O., Selvi Y., Ozkol H., Tuluce Y., Besiroglu L. and Aydin A. (2012). Comparison of superoxide dismutase, glutathione peroxidase and adenosine deaminase activities between respiratory and nocturnal subtypes of patients with panic disorder. Neuropsychobiology, 66:244-251.