

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

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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 pathophysiology 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 effect 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 who 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 methylprednisolone (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 (H₂O₂) 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 Methylprednisolone showed a better results of adenosine

deaminase (ADA) specific activity than treating with IFN-β this could be proved that the Methylprednisolone 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 ≤ 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 ammonia (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.

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