Effect of Methotrexate on Adenosine Deaminase Activity in Rheumatoid Arthritis Iraqi Patients

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Abstract

Rheumatoid arthritis (**RA**) is considered as an autoimmune disease which influences the joints. It usually results in a warm, enlarged, and painful joints. Stiffness and Pain typically amplify once relaxation. Frequently, the hands and wrist are involved, with similar joints naturally involved on both sides of the body. The syndrome might additionally have an impact on the other parts of the body. Adenosine Deaminase is an enzyme (EC 3.5.4.4) used with the metabolism of purine. It is essential for the analysis of adenosine from nutrition and the revenue of nucleic acids in soft tissue. Its chief purpose in humans is the immune system growth and preservation. On the other hand, the complete physiological role of ADA is so far not known. This study carried out at Al-Kadima Teaching Hospital, during the period fr6-om September 2014 till the end of October 2015. Patients who have any other disorders conditions are excluded. Subjects accepting treatment with medications within 7-9 months. A total of 66 patients with Rheumatoid arthritis were enrolled in this study: thirty-five of them were newly diagnosed to have Rheumatoid arthritis who receives no therapy for Rheumatoid arthritis (group A); the remaining thirty-one patients were with Rheumatoid arthritis who receive by methotrexate 500 mg for 7-9 months as the (group B). Twenty-six healthy subjects, as controls(group C). The results show a significant elevation in the ADA activity in group A and group B. The study concluded that the level of ADA was higher in Iraqi RA patients than in healthy control subjects

Keywords: Rheumatoid arthritis, Adenosine Deaminase and methotrexate

Introduction

Rheumatoid arthritis (**RA**) is considered as an autoimmune disease which influences the joints. It typically results in warm, inflamed, and hurting joints. Rigorousness and Pain characteristically amplify once relaxation. Frequently, the hands and wrist are involved, with the analogous joints certainly intricate on both sides of the body. This force clue to inflammation in the heart, inflammation in the lung, and low red blood cell count, fatigue and elevated in the body temperature could also be found.(1)

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Recurrently, signs arise on gradually from weeks to months. Even though the fact that the reason for rheumatoid arthritis is not well identified, it is speculative to contain a miscellaneous of environmental factors and genetic. The exceptional mechanism comprises the aggressive action of the immune system of the body which indication to solidifying and irritation of the joint. It influences cartilage and bone.[1]

The sympathy of this syndrome is to be influenced by a person's symptoms and signs.(2) RA affects between 5 and 50 per 100,000 people newly developing the condition and between 0.5 and 1% of adults in the developed world each year.(3) Onset is persistent through middle age female are influence 2.5 times as often as men.[1] In 2013, it gives rise to in 38,000 deaths up from 28,000 deaths in 1990.(4) Adenosine Deaminase is an enzyme (EC 3.5.4.4) used with the metabolism of purine. It is essential for the breakdown of adenosine

from sustenance and for the revenue of nucleic acids in soft tissue. Its topmost purpose in humans is the immune system growth and preservation.[5] Instead, the complete biological character of ADA is not recognized ADA present in both major form (dimer-complex) and minor form (monomer). [6] In the monomer configuration, the enzyme is a polypeptide chain,[7] fixed into eight elements of equivalent α/β barrels, which frame a topmost deep pocket that is the active site. Along with the eight essential β-barrels and eight marginal α-helices, ADA likewise embraces five further helices: residues 19-76 fold into three helices, located between β1 and α1 folds; and two antiparallel carboxy-terminal helices are placed across the amino-terminal of the β-barrel. The ADA active site involves a zinc ion, which is located in the deepest recess of the active site and synchronized by the substrate and five atoms from His15, His17, His214, Asp295.[5] The substrate is bound to an active site by nine hydrogen bonds and become stable. The carboxyl group of Glu217, approximately coplanar with the substrate purine ring, is in the site to formulate a hydrogen bond with N1 of the substrate. The carboxyl group of Asp296, correspondingly coplanar with the substrate purine ring, forms a hydrogen bond with N7 of the substrate. The NH group of Gly184 is in position to consist of a hydrogen bond with N3 of the substrate. Asp296 forms bonds composed with the Zink ion (Zn+2) laterally with 6-OH of the substrate. His238 also hydrogen bonds to substrate 6-OH. The 3'-OH of the substrate ribose figure a hydrogen bond over and done with Asp19, conversely the 5'-OH construct a hydrogen bond through His17. Double extra hydrogen bonds are made to water particles, at the preliminary of the active site, thru the 2'-OH and 3'-OH of the substrate. In place of the importance of the recessing of the active site in the interior enzyme structure, the substrate, immediately it bound, is very nearly completely recaptured from the solvent.[7] The surface area of the substrate to solvent once bound is 0.5% the surface area of the substrate in the Free State.

Material and Methods

Subjects: the study was a cross-sectional study carried out at Al-Kadima Teaching Hospital, during the period from September 2014 till the end of October 2015. Patients who have any other disorders conditions are excluded. Subjects accepting treatment with medications within 3 months. All subjects were nonsmokers, and none reported chronic alcohol consumption.

The protocol for the study was approved by the Ethical committee of Al-Nahrain Medical College and informed signed consent was given by each subject. A total of 66 patients with Rheumatoid arthritis were enrolled in this study: thirty-five of them were newly diagnosed to have Rheumatoid arthritis who receives no therapy for Rheumatoid arthritis (group A); the remaining thirty-one patients were with Rheumatoid arthritis who receives by methotrexate 500 mg 7-9 months as a therapy (group B). The study included another Twenty-six healthy subjects, non- alcoholic, non-smoking and no family history of any type of Diabetes Mellitus or Hypertension who serve as healthy controls(group C); they were matched with patients` groups for age as in table 1

Blood samples:

Five millilitres of random venous blood was withdrawn from each patient, in the supine position, without application of a tourniquet. Samples were transferred into a clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendorf tube and was used for measurement of ADA. The tubes were stored at –20 ºC until analysis, which was done within one month after collection.

Procedure: measurement of serum ADA was done by ELISA kit [8].

Statistical analysis: statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significance of the mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results

Table (1) clinical criteria of patients groups with Rheumatoid Arthritis and control (mean ±sd)

Parameters	Group A	Group B	Group C
Age rang (years)	45-67	45-67	45-67
ADA (IU/l)	13.248±4.145	32.268±4.779	18.738±4.34

Table (2) p-value between the studied groups

The Groups	Group A	Group B	Group C
Group A	--------	≥0.001	≥0.001
Group B	≥0.001	≥0.001	≥0.001
Group C	≥0.001	≥0.001	≥0.001

Discussion

The preclusion of joint damage and reducing the sickness form vital mechanisms of administration of RA patients, the first establishment of the appropriate treatment is essential. Even though clinical guides help in appraising syndrome activity, biochemical indicators are furthermore one of the things for signs of syndrome activity and progress. This study demonstrated in table 1 group B has the maximum level of ADA, though group A the lowest level of ADA. Nevertheless, the strict source of flow in ADA levels is not recognized, the activity might be greater than before as a consequence of its ejection from the damaged cells and initiate of cellular proliferation in RA [(9)] Nalesnik M *et al.,* revealed an insignificant association between CRP and ADA in patients with RA without methotrexate treatment and recommended that ADA activity in serum might be used as a biochemical indicator of the inflammatory progression in RA [(10)].

ADA catalyzes the irremediable hydrolysis of adenosine to inosine. Adenosine has been revealed to be a strong endogenous anti-inflammatory agent [(11).] The enzyme ADA characterizes a checkpoint in the directive of extracellular Adenosine levels [(12)] and therefore is possible to moderate the inflammatory procedures.

A study by Sari et al., [(13)] exposed that serum total ADA activity is linked with RA and may propose a respected helper to measure inflammation moreover to the traditional indices. That suggested that determining ADA activity help for a superior clarifying of some of the pathophysiological sorts of the disease.

Methotrexate is the anchor drug for remedy of RA. Subsequently, the anti-inflammatory influence of MTX is through the incitement of adenosine receptors, it was reflected that the therapeutic properties of MTX could be as a result of catalytic activity of ADA in the serum [(14)] The pathophysiology of adenosine ejection is associated with MTX pathways. It is recommended that MTX stops ADA and surges vasodilatation convinced by adenosine so it is a deduction that ADA activity in serum may be an imaginable biochemical indicator for checking the therapeutic properties of MTX in RA patients. Van Ede et al. resolved a connection between ADA and MTX treatment in patients receiving MTX and folic acid [(15)]. Salesi et al. revealed that ADA levels earlier and later the MTX treatment in their RA group and point to a connotation with ADA levels with the usefulness of MTX treatment [(16)]

Conclusion

This study showed that level of ADA was higher in

Iraqi RA patients than in healthy control subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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