

## Original Article

### Serum adenosine deaminase in patients with rheumatoid arthritis treated with methotrexate

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#### ABSTRACT

**Objective:** Recently, adenosine deaminase (ADA) is introduced as helpful marker in diagnosis, prognosis, and monitoring of treatment in rheumatoid arthritis (RA). The aim of this study was to determine the efficacy of the serum ADA in diagnosis, prognosis, and monitoring of treatment with methotrexate (MTX) in RA.

**Methods:** This was a self-controlled clinical trial conducted in university hospitals of Isfahan, Iran. The serum level of ADA, erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF) were measured for 70 patients with active RA (Disease Activity Score-28 [DAS28] > 3/2). After three months of MTX treatment and disease remission (DAS28 < 2.6) these markers were measured again. ANCOVA multiregression and paired *t*-test were used to compare and evaluate the mean level and correlation of ADA, ESR, IgM-RF, and DAS before and after RA remission.

**Findings:** The mean value for ADA activity was significantly higher than the normal one compared with other studies. Significant decreases were seen in values of ADA, ESR, RF, visual analogue scale (VAS), and DAS after remission. Also, the correlation coefficient between the values of ADA with ESR and DAS were statistically significant in baseline. Moreover, the statistically significant correlation between ADA and ESR, VAS, and DAS were seen after remission. No correlation was found in the case of the dosage of MTX with the value of ADA.

**Conclusion:** It was concluded that ADA may be considered useful as a marker in diagnosis, prognosis, and monitoring of treatment with Methotrexate in RA.

**Keywords:** Adenosine deaminase; disease activity score; methotrexate; rheumatoid arthritis

#### INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease which usually affects the joints of hands and feet as an erosive, symmetrical polyarthritis. Although the cause of rheumatoid arthritis is unknown, it is considered as an autoimmune disorder involving other joints and organs. The worldwide prevalence

of RA was reported as approximately 1%, while it is more common in 7<sup>th</sup> decade of the life.<sup>[1,2]</sup>

The pathogenesis of RA is based on the impaired function of immune system. Although rheumatoid arthritis was mainly thought as a T cells-mediated disease, it is characterized by accumulation of chronic inflammatory cells including T and B lymphocytes, monocytes, and macrophages. T cells (CD4+) activate B cells and macrophages via cell-surface receptors and secrete cytokines such as interferon (IFN) and interleukin (IL-17). Also, plasma cells develop from B cells while neutrophils and macrophages are attracted by macrophage released cytokines (TNF  $\alpha$ , IL-1).<sup>[1]</sup>

RA diagnosis is clinical. Physicians use American College of Rheumatology criteria for the diagnosis of RA. However, such criteria have some limitations in

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early RA diagnosis. Additionally, the autoantibodies and high level of inflammatory markers help in diagnosis while they are also important for assessment of disease activity and monitoring of treatment.<sup>[3]</sup> Not only the number of swollen and tender joints, but also the biologic markers can be indicator of disease activity and progression. Such biomarkers include the level of acute-phase reactants and the laboratory markers such as rheumatoid factor (RF) and anti-citrulline antibodies. Furthermore, the genetic markers like polymorphisms in HLA-DR or candidate genes can be helpful in this regards.<sup>[4]</sup>

To assess the disease activity, there is the Disease Activity Score (DAS) and its modifications such as the DAS28 (based on 28-joint counts), the DAS28-CRP, the Simplified Disease Activity Index (SDAI), and Routine Assessment of Patient Index Data (RAPID). However, their use has some limitations such as complexity and difficulty in calculation. Therefore, they are not useful for immediate decision making.<sup>[5,6]</sup>

As it was mentioned, the levels of acute phase reactants such as CRP and ESR are the best predictors of RA progression. This is because they are the biomarkers of pro-inflammatory cytokine production. While ESR is just useful for evaluation of disease activity of the past few weeks, CRP is more beneficial for estimating short-term changes in disease progression.<sup>[7,8]</sup>

On the other hand, adenosine deaminase (ADA), one of the main enzymes of the purine metabolic pathway, has been considered as a marker of cell mediated immunity. It is present in most tissue as well as serum and it is one of the main components for maturation and function of lymphocytes and formation of macrophages from monocytes. As a result of cell-mediated immune response in RA patients, the activity of ADA, which catalyze the deamination of adenosine to form inosine, elevate.<sup>[8,9]</sup>

Moreover, since the anti-inflammatory effect of methotrexate (MTX), one of the drugs in RA therapy, is via the stimulation of adenosine receptors, it was considered that the therapy effects of MTX could be as a result of catalytic activity of ADA in serum.<sup>[3]</sup> Therefore, it seems that the serum level of ADA can be used as a useful parameter in both estimating the prognosis of disease and monitoring the therapeutic effect of MTX.<sup>[3,9-11]</sup> The aim of this study was to find the efficacy of the serum level of adenosine deaminase in diagnosis, prognosis, and monitoring of treatment with methotrexate in RA patients.

## METHODS

This multicenter self-controlled clinical trial was performed in university hospitals of Isfahan, central

of Iran. This prospective study was conducted between May 2011 and January 2012. The minimum sample size for this study was calculated as 70, although 66 of them completed the study. Four patients did not come to give the follow up blood tests after three months. Eligible participants were patients with definite diagnosis of RA using American College of Rheumatology criteria who also have active RA (DAS 28 > 3/2). Ones having RA overlapped with other rheumatoid disease were not included. Ethical approval based on the Declaration of Helsinki was obtained from the Isfahan University of Medical Sciences Ethics Committee and the written informed consent was taken from all patients.

Ten milliliter of venous blood was taken by arm venous puncture in sterile vials. 8ml of blood collected without anticoagulant. Serum was separated by centrifugation at 3500 rpm for 20 mins and was used for measurement of ADA and IgM-RF. Serum ADA was measured by adenosine deaminase (ADA) ELISA Kit (Orgentec Diagnostika, Mainz, Germany). Also, the serum level of IgM-RF was measured by ELISA (Orgentec Diagnostika, Mainz, Germany). The remaining of blood sample was collected in sterile tube containing potassium-EDTA anticoagulant for measurement of ESR by Westergren method.

Then, the patients were given the MTX or the dosage of the medication was increased if they had been given the medication before the commencement in the study. The treatment period lasted for three months and after the disease remission (DAS28 < 2.6), the serum activity of ADA, IgM-RF, and ESR were measured again at the end of the study. Additionally, the dosage of the MTX, Visual Analogue Scale (VAS), and Disease Activity Score (DAS) were measured and recorded for patients at baseline and end of the study.

Data were analyzed with SPSS software version 15 (SPSS® Inc.) using Ancova multiregression and paired *t*-test. The relation between ADA activity and Disease Activity Score (DAS28), Visual Analogue Scale (VAS), ESR, and IgM-RF were evaluated before and after RA remission. A power of 80%, a one-tailed 5% significance level, and dropout rate of 10% were considered for this study.

## RESULTS

Form 66 patients, 57 (86.4%) of them were women while only 9 (13.6%) of who were men. The mean age of the patients was  $49.47 \pm 15.0$  ( $48.4 \pm 14.3$  for women and  $56.2 \pm 18.4$  for men). Also, changes in ADA activity were not different in males and females (*P* value = 0.41).

Table 1 shows ADA activity and some other

**Table 1: Adenosine deaminase activity and some laboratory parameters measured for the patients at baseline and after remission**

| Measured parameters         | Baseline (Mean±SD) | Remission (Mean±SD) | P value |
|-----------------------------|--------------------|---------------------|---------|
| Adenosine deaminase (U/Lit) | 39.9±14.6          | 34.9±13.2           | 0.02    |
| Rheumatoid factor (IU)      | 51.6±29.9          | 36.0±23.3           | <0.001  |
| ESR (mm/h)                  | 34.8±18.2          | 16.7±11.9           | <0.001  |
| Tender joint count          | 5.3±4.2            | 0.2±0.6             | <0.001  |
| Swollen joint count         | 3.9±3.6            | 0.8±1.2             | <0.001  |
| VAS (0-100 mm scale)        | 33.9±20.1          | 6.2±8.6             | <0.001  |
| DAS                         | 4.5±1.1            | 2.1±0.5             | <0.001  |

ESR: Erythrocyte sedimentation rate, VAS: Visual analogue scale, DAS: Disease activity Score

measured parameters at the baseline and after three months of treatment with methotrexate (MTX) (after remission). A significant decrease were seen in value of ADA, ESR, RF, VAS, DAS, and the number of tender joints, and swollen joints comparing before and after remission [Table 1].

The determined correlation coefficient between the values of ADA catalytic activities and other measured parameters before remission are demonstrated in Table 2. The determined correlation coefficient between the values of ADA with ESR and DAS were statistically significant in baseline. In Table 2 such correlations are shown after remission as well. The statistically significant correlation between ADA with ESR, swollen joints, VAS, and DAS were seen while no correlation was found in the case of the dosage of MTX with the value of ADA.

## DISCUSSION

Since preventing RA progression and decreasing the mortality and morbidity of RA is a crucial point in management of such patients, it is necessary to initiate the aggressive therapy as soon as possible. Although RA diagnosis is based on the clinical findings and ACR criteria, much more applicable markers and criteria are felt to be needed for early diagnosis and difficult differential diagnosis of RA and other autoimmune connective tissues diseases.<sup>[3]</sup>

The normal range reference for ADA activity is assumed as 14-22 mmol/L.<sup>[12]</sup> Additionally, different studies have found different values for normal human serum ADA level at 37°C. It was 19.65 ± 3.56 U/Lit, 15.7 ± 7.1 U/Lit, 5.9 ± 17.6 U/Lit, 12.49 ± 2.50 U/Lit, 13.14 ± 4.28 U/Lit, 15.8 ± 3.7 U/Lit, 17.05 ± 3.75 U/Lit, 19.09 ± 2.99 U/Lit, and 15.30 ± 0.2290 U/Lit<sup>[3,9,13]</sup> The mean value for ADA activity in our patients was 39.9 ± 14.6 which is significantly higher than the normal ones compared with other studies (39.9 ± 14.6 vs. 19.65 ± 3.56, *P* value = 0.03). Moreover, our result

**Table 2: Correlation between the values of ADA catalytic activities and other measured parameter before and after remission**

| Measured parameters  | Adenosine deaminase activity |         |                 |         |
|----------------------|------------------------------|---------|-----------------|---------|
|                      | Before remission             |         | After remission |         |
|                      | r                            | P value | r               | P value |
| ESR                  | 0.203                        | 0.05    | 0.235           | 0.02    |
| Rheumatoid factor    | -0.087                       | 0.24    | 0.074           | 0.28    |
| Tender joint count   | 0.052                        | 0.34    | 0.066           | 0.30    |
| Swollen joint count  | 0.095                        | 0.22    | 0.201           | 0.05    |
| VAS (0-100 mm scale) | 0.121                        | 0.16    | 0.213           | 0.04    |
| DAS                  | 0.213                        | 0.04    | 0.241           | 0.02    |
| Methotrexate usage   | -                            | -       | -0.058          | 0.32    |

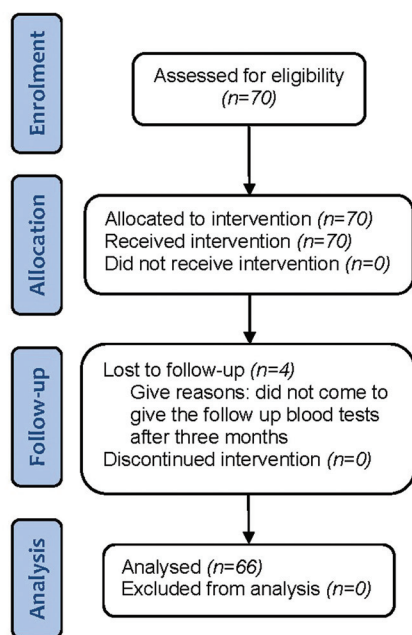
ESR: Erythrocyte sedimentation rate, VAS: Visual analogue scale, DAS: Disease activity Score

showed that the mean value of ADA, ESR, RF, VAS, and DAS were significantly decreased after treatment with MTX.

At baseline, significant correlations were observed between serum ADA level and ESR which is a marker of disease activity. In addition, efficacy of MTX was measured after three months of treatment. Changes in enzyme activities of ADA showed significant correlation with ESR, the number of swollen joints, VAS, and DAS after treatment. Therefore, the change in ADA enzyme activity was associated with the efficacy of MTX treatment. However, no correlation was found between the dosages of MTX with serum level of ADA after remission.

The increased serum level of ADA is indicator of stimulation of cellular immunity. For instance, this condition can be seen in lymphoblastic leukemia, acute hepatitis, human immunodeficiency virus infection, infectious mononucleosis, tuberculosis, pneumonia, and rheumatoid arthritis. It was claimed that the major source of the prevalent form of ADA (ADA2) in serum is the monocyte/macrophage cell system.<sup>[12,14]</sup> The raise in serum ADA level in RA patients can be explained by the immunity status changes. In this case, the ADA level reflects the monocyte/macrophage activity or turnover.<sup>[15]</sup> Many studies demonstrated the elevated serum level of ADA in RA patients.<sup>[3,10,14-18]</sup>

In comparison of serum ADA of healthy subjects with two groups of RA patients, it was shown that serum ADA of RA patients not receiving MTX is significantly higher than healthy people while in contrast, it was significantly lower in RA patients under treatment with MTX.<sup>[3]</sup> In another study conducted in Turkey, total serum ADA activity of RA patients were significantly higher than control group and a strong correlation was found between the serum level of this enzyme and disease activity by DAS. However,



The CONSORT diagram of the clinical trial

no correlation was found between ADA and CRP or ESR in case groups.<sup>[10]</sup> Moreover, Surekha Rani *et al.* demonstrated the importance of ADA as a marker of rheumatoid arthritis diagnosis since the mean level of ADA was  $59.79 \pm 21.09$  in patients which is significantly higher than  $20.71 \pm 5.63$  in healthy group.<sup>[15]</sup> In this study the C-reactive protein level was significantly higher in RA patients, which is an indicative of active inflammation. Additionally, Pallinti *et al.* showed the importance of some serum biochemical markers including adenosine deaminase, malondialdehyde, and homocysteine in diagnosis of RA, whereas no correlation was found between these markers and markers of disease activity, such as ESR and CRP. It means that the contribution of these biochemical markers in disease process is completely independent.<sup>[16]</sup>

It is proposed that MTX works in different ways to decrease the inflammation. It stimulates the adenosine receptors, increases vasodilation, inhibits ADA, decreases RF, IL-6, IL-8, receptors of TNF- $\alpha$ , and IL-2.<sup>[3]</sup> Although, the exact effect of MTX on the ADA enzymatic activity is not clearly understood, it is assumed that in purine enzyme metabolic pathway, MTX increases adenosine and inhibits ADA both directly and indirectly. In addition, the decrease of ADA leads to adenosine rise which has been introduced as an endogenous anti-inflammatory agent.<sup>[18,19]</sup> In our study the decline of ADA was observed after treatment with MTX. Same results were also demonstrated in another study by van Ede *et al.* They showed the increased level of some of the purine enzymes pathway, including ADA, and the

decreasing trend of such enzyme during treatment with methotrexate. However, they demonstrated no correlation between such decreasing trend and the efficacy of MTX.<sup>[18]</sup>

This study is among the first studies which evaluate the serum level of ADA during MTX treatment. However, one of the drawbacks of this study was having no control group to compare the result of the RA patients and achieve much more precise results and conclusion. To overcome this problem, the result of this study was compared with the control groups of other studies to make such limitation less important.

In conclusion, this study demonstrated the elevated level of ADA in RA patients which can be used as a helpful marker for diagnosis of this disease. Also, the decline of ADA enzyme activity throughout MTX treatment of patients with RA was seen which was in association with the efficacy MTX treatment. Additional studies are required to find out the exact role of purine metabolism in RA and in treatment with MTX. Larger studies with control group are needed to find the exact mechanism of MTX on ADA enzymatic activity and adenosine and the role of ADA metabolism in RA. Also, it is suggested to measure the ADA isoenzymes in future studies to determine if MTX has any specific effect on one of these isoenzymes.

## AUTHORS' CONTRIBUTION

All authors had active contributions in all stages of this study.

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