Correlation of serum levels of adenosine deaminase activity and its isoenzymes with disease activity in rheumatoid arthritis

R.A. Sari¹, S. Taysi², Ö. Yilmaz¹, N. Bakan²

¹Department of Immunology/Rheumatology; ²Department of Biochemistry, School of Medicine; ¹Internal Medicine, Atatürk University, Medical School, Erzurum, Turkey.

Abstract

Objective. Adenosine deaminase (ADA) is involved in purine metabolism and plays a significant role in the mechanisms of the immune system. The aim of this study was to investigate the correlation between the activity of total ADA (tADA) and isoenzymes ADA1 and ADA2 and clinical activity in patients with rheumatoid arthritis (RA).

Methods. The study comprised 37 patients with rheumatoid arthritis and 30 healthy controls. Total serum ADA activity was measured spectrophotometrically. The isoenzyme pattern of ADA was analyzed using erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). Disease activity was assessed using DAS criterion.

Results. The means of tADA, ADA1 and ADA2 levels were found to be 22.7 ± 5.9, 3.9 ± 2.0 and 18.8 ± 4.9 IU/L in patients with RA, and 15.1 ± 4.4, 4.1 ± 1.2 and 11.0 ± 3.7 IU/L in healthy control subjects. When compared to the healthy controls, serum tADA and ADA2 levels were significantly higher (p < 0.001) in patients with RA, but the decrease of ADA1 level was not statistically significant (p > 0.05). There was a strong correlation (r = 0.527, p < 0.001) between serum tADA level and disease activity by DAS (Pearson’s rank correlation), and no correlation between tADA and CRP or ESR in the patient groups (p > 0.05).

Conclusion. Serum tADA and ADA2 activity is closely associated with RA and these non-invasive investigations can be used as biochemical markers for inflammation. These may provide additional information regarding disease activity along with the traditional indices such as ESR and CRP.

Introduction

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis, sometimes with multisystem involvement (1, 2). It affects about 1% of the population world-wide and may lead to progressive joint destruction, deformity, disability and premature death (1-3).

Most RA patients exhibit clinical activation and remissions. Laboratory as well as clinical findings play an important role in determining disease activity. As in other collagen tissue diseases such as systemic lupus erythematosus, among laboratory tests intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), selectins, adenosine deaminase (ADA), and cytokine deaminase are used together with such routine analyses as ESR and CRP to determine activity in RA (4).

ADA, an enzyme involved in purine metabolism or the purine salvage pathway, catalyses the irreversible conversion of (deoxy) adenosine to (deoxy) inosine. ADA is found widely in most mammalian tissues, showing the highest activity in lymphoid tissues, and is essential for the proliferation, maturation, and function of lymphocytes (5), especially those of T lineage; it is also required for the maturation of human blood monocytes to macrophages (6).

Adequate ADA levels appear to be necessary for normal functioning of the immune system (7). Since ADA activity has been shown to be increased in diseases characterized by T lymphocyte proliferation and activation (8), ADA has been considered as a non-specific marker of T cell activation. It has become increasingly evident that adenosine plays an important role in the regulation of immune responses. Numerous reports have pointed to the general immunosuppressive and anti-inflammatory properties of this compound (9, 10), and support the view that adenosine acts as an immunosuppressive agent, which may have relevance for both cell-mediated and inflammatory immune responses (11).

Two isoenzymes of adenosine deaminase, adenosine deaminase 1 (ADA1) and adenosine deaminase 2 (ADA2) have been described in many of the higher organisms (12, 13). ADA1 is present in all tissues and is essential for an efficient immune response. While in human tissues and cells the majority of ADA activity is derived from ADA1, the prevalent form of ADA in serum is ADA2. Recent data suggest that the monocyte-macrophage cell system is likely to be a major source of ADA2 in humans (14).

Several studies have been performed...
correlating ADA activity to immune status in various chronic liver diseases, hematologic malignancies, and immune disorders (15-18). However, serum ADA activity has been described in various autoimmune and inflammatory diseases: rheumatoid arthritis (19), systemic sclerosis (20) and systemic lupus erythematosus (12).

To our knowledge, there have been no studies focused on measuring serum tADA, ADA1, ADA2 activities and DAS in patients with RA. Therefore, in the present study we aimed to investigate serum tADA, ADA1, ADA2 activities and to correlate their DAS with RA.

Materials and methods

Thirty-seven patients suffering from RA for 2-10 years (14 men, 23 women with a mean age of 52 ± 11 years) and 30 healthy subjects (14 men, 16 women, with a mean age of 40 ± 12 years) were recruited for the study after obtaining their informed consent. The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. All the patients examined met the American Rheumatism Association criteria for RA (21) and were taking no drugs. None of the subjects had any alcohol intake, intestinal absorption defect, or any clinical or laboratory signs of liver disease, diabetes mellitus, thyroid disease, infectious disease, or coronary artery disease. Control subjects were healthy medical students, laboratory personnel and those attending a health screening program.

The erythrocyte sedimentation rate (ESR) was determined according to the Westergreen method using anticoagulant containing whole blood. Venous blood was collected in vacutainers without additive, allowed to clot for 30 min at room temperature and centrifuged at 3000 g for 5 min. to obtain serum. The serum aliquots were stored at -80°C until the biochemical analyses were carried out. Hemolysed samples were excluded.

The serum C-reactive protein (CRP) level was determined by the nephelometric method (Beckman Array 360 Protein System, USA).

All enzymes activities were measured spectrophotometrically (22,23). ADA2 activity was determined by a modified method of Hartwick et al. using erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) (24), which specifically inhibits only ADA1. Biochemical measurements were carried out at room temperature using a CE 3041 spectrophotometer (Cecil, UK). The disease activity score (DAS) was calculated using the following formula as described (25, 26): DAS = 0.53938 x RAI + 0.06465 SW + 0.330 x ln ESR + 0.224 where RAI is the Ritchie articular index, SW the number of swollen joints, and ESR the erythrocyte sedimentation rate.

Statistical analysis

The Mann-Whitney U test and Pearson’s rank correlation test were used for statistical evaluation. P < 0.05 was considered significant. SPSS for Windows (version 10.0) was used for statistical analyses.

Results

The means of tADA, ADA1 and ADA2 levels were found to be 22.7 ± 5.9, 3.9 ± 2.0 and 18.8 ± 4.9 IU/L in RA patients, and 15.1 ± 4.4, 4.1 ± 1.2 and 11.0 ± 3.7 IU/L in healthy control subjects, respectively (see Table I). When compared to the healthy control subjects, serum tADA, ADA2 were significantly higher (p < 0.001) in RA patients, but the decrease of ADA1 level was not statistically significant (p > 0.05).

There was a strong correlation (r = 0.527, p < 0.001) between serum tADA activity and DAS. Correlations were also found (r = 0.397, p < 0.05) between ADA1 activity and disease DAS, and (r = 0.462, p < 0.005) between ADA2 activity and DAS. No correlations were found between tADA and CRP, ESR in the patient groups (p > 0.05) (Fig. 1).

Discussion

The etiology of rheumatic diseases such as RA is still unknown, although many possible causes, including infectious, genetic, immunologic etc., have been investigated (27). Juvenile rheumatoid arthritis (JRA) is characterized by involvement of multiple systems or organs and by the appearance of immunological abnormalities such as the production of a variety of autoantibodies, the presence of circulating immune complexes, and disruption of T-cell/B-cell interactions (28). Activated macrophages and T cells have been implicated in mediating the initiation and perpetuation of inflammation in JRA and SLE. These cells are responsible for the immunoregulatory and inflammatory response (29).

Serum ADA2 activity probably reflects macrophage activation in addition to PBLs activation as part of a cellular immune response (30,31). ADA2 is actively secreted by monocytes or certain subpopulations of monocytes with a different isoenzyme profile, or the lifetime of ADA2 in serum may be longer than that of ADA1 (32). Therefore, the measurement of ADA2 activity is important, because ADA2 increases with the advancing clinical grade of the disease (29).

While ADA1 has been extensively stu-

Table I. The mean values of serum tADA, ADA 1, ADA2, ESR, CRP, and DAS levels in healthy and RA groups.

<table>
<thead>
<tr>
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<th>Healthy group (n=30)</th>
<th>Rheumatoid arthritis group (n=37)</th>
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<tbody>
<tr>
<td>tADA (IU/L)</td>
<td>15.1 ± 4.4</td>
<td>22.7 ± 5.9*</td>
</tr>
<tr>
<td>ADA1 (IU/L)</td>
<td>4.1 ± 1.2</td>
<td>3.9 ± 2.0</td>
</tr>
<tr>
<td>ADA2 (IU/L)</td>
<td>11.0 ± 3.7</td>
<td>18.8 ± 4.9*</td>
</tr>
<tr>
<td>ESR* (mm/h)</td>
<td>11.6 ± 4.6</td>
<td>51.9 ± 18.3*</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.5 ± 1.5</td>
<td>19.5 ± 8.5*</td>
</tr>
<tr>
<td>DAS*</td>
<td>NA</td>
<td>3.38 ± 1.5</td>
</tr>
</tbody>
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*p<0.001, when compared to control group.
*ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; DAS: Disease activity score (see Materials and Methods); NA: not applicable.
died and characterized, the biochemistry and physiology of ADA2 is poorly understood (12). It was attempted to study the purine metabolism of JRA patients by measuring tADA and isoenzyme ADA1 and ADA2 activity in the serum of these patients in different phases of the diseases. The serum tADA and ADA2 activity in this study was significantly higher in the sera of patients with JRA during disease activity than in those of healthy controls (29).

We determined disease activity taking into consideration the clinical findings (DAS) and laboratory findings (CRP, ESR) in our study. We found a significant positive correlation between serum tADA, ADA2, and ADA1 levels and DAS criterion. However, we could not find any positive correlation between ESR and CRP levels and DAS in these patients. In the literature, activity in RA patients has been evaluated according to the clinical and, above all laboratory findings of the patients.

The origin of tADA in serum, the mechanism and etiology by which its activity increases have not been fully elucidated. Possibly, the increased serum tADA activity in RA can be correlated with the immune response (33).

To determine disease activity in RA patients, only a certain number of studies have been carried out. Nalini et al. (31), Ungerer et al. (32), Cordero et al. (34), and Hitoglou et al. (29) have studied tADA and ADA2 activity in RA patients, and found a positive correlation between disease activity and ADA activity. In all of these studies, a positive correlation was established between disease activity and tADA and ADA2 activity. These results are similar to those of our study.

The exact cause of the elevated serum ADA in RA was not established in this study, but the possibility exists that the enzymes released into the circulation from damaged cells are associated with cellular proliferation and increased turnover of the cells involved (31). Stancikova et al. (12) and Taysi et al. (35) have studied ADA activity in patients with SLE, and found that serum tADA and ADA2 isoenzymes increased considerably in patients with SLE compared with healthy controls. Recently, more attention has been paid to extracellular adenosine, the substrate of ADA, because it is a potent endogenous antiinflammatory agent that inhibits neutrophil function, is released by cells under metabolically unfavorable conditions, and that this mechanism is stimulated by methotrexate, the antiinflammatory agent for RA. Thus it is important to clarify the clinical significance of ADA isoenzyme activities in RA (36).

In the light of these results we would propose that serum tADA activity, specifically ADA2 and ADA1 activity, may be a useful adjunct to the present enzyme repertoire in clinical medicine (7). These non-invasive investigations can be used as biochemical markers for inflammation and may provide additional information regarding disease activity alongside the traditional indices such as ESR and CRP (13).

In addition, because of its low cost and easier application, the measurement of plasma ADA activity may be clinically useful for predicting relapses before clinical findings and also for following up the disease during the treatment period in RA patients, after further studies of its sensitivity have been made (5). Therefore, it is important to clarify the clinical significance of ADA isoenzyme activities in RA.

References
8. COLLAZOS J, ESPANA P, MAYO J, MARTINEZ E, IZQUIERDO F: Sequential evalua-