Adenosine deaminase and Guanase deaminase activities in serum of patients with rheumatoid Arthritis

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List of abbreviations
AD, adenosine deaminase
GD, guanosine deaminase
RA, rheumatoid arthritis
ROS, reactive oxygen species

Abstract
We investigated purin and primidin salvage pathways in rheumatoid arthritis (RA). For the aim, Adenosine deaminase (AD) and Guanosine deaminase (GD) activities in serum of patients with different manifestations of the disease (n=32) were investigated and compared with the control group of healthy individuals (n=52). RA cases were classified with respect to their clinics and treatment.

The ADA activities were significantly higher (p<0.01) in patients with RA than in control although GD activity levels were found to be lower (p<0.01). When compared their AD and GD levels amongst the classified RA groups, there was no difference in AD levels amongst cases.

In conclusion, increased serum AD activities in patients with RA may be dependent on and reflect the increase in phagocytic activity of macrophages and maturation of T-lymphocytes. The results indicated that plasma AD and GA activity can be used for the diagnosis of RA disease to support clinical findings and as an index for disease.

Keywords
Rheumatoid arthritis, Adenosine deaminase, Guanosine deaminase, Inflammation
Role of AD and GA activities in serum of patients with Rheumatoid Arthritis

Introduction

Adenosine deaminase (AD), adenosine aminohydrolase, (E.C.3.5.4.4.) is an enzyme involved in the catabolism of purine bases, capable of catalysing the deamination of adenosine, forming inosine in the process (Fox and Kelly 1978, Van Linde and Eitzschig, 2007). The AD and Guanosine deaminase (GD) are two enzymes that is required for lymphocyte proliferation, maturation and differentiation with detected biologic activity, particularly in T cells (Bukulmez et al. 2000). AD and GD activities are known to be increased in inflammatory diseases characterized by T-cell activation and proliferation. Therefore, AD and GD is considered a marker of T-cell activation. Hence, its main physiologic activity is related to lymphocytic proliferation and differentiation. As a marker of cellular immunity, its plasma activity is found to be elevated in diseases in which there is a cell-mediated immune response (Erkiliç et al. 2003).

The mechanism(s) by which immune components play role in RA pathogenesis has been the subject of intense research in recent years. The onset of RA can vary among individual patients (Yazar et al. 2005). The earliest alterations relate to the vasculature, with vascular congestion and even obliteration of small vessels by inflammatory cells and thrombi. In addition to this microvascular injury, hyperplasia and hypertrophy of the synovial lining cells and a modest perivascular accumulation of leukocytes are also typical findings (Klarenbeek et al. 2009). The chronic phase of RA is grossly characterized by edema and swelling of the synovial. While polymorphnuclear leukocytes are dominant in the synovial fluid at onset, the extra-vascular lymphocytic infiltration becomes more abundant in the chronic phase. They consist largely of CD4+ helper T cells, which are in close opposition to antigen presenting cells. Pathologically, this is quite similar to the characteristics delayed-type hypersensitivity (Le et al. 2007; Katchamart et al. 2010). Although several pathogenic molecules have been implicated, the intermittent nature and the lack of consistent response to therapy make the underlying etiology difficult to define. Diagnosis is usually based its clinical presentations and there is, however, no any specific laboratory test to make the diagnosis. It is therefore of great importance to determine the factor(s) that may lead to disease and to find some rapid and useful laboratory analysis for the diagnosis of RA disorder.

To test the inter-relationship between T-cell hyperfunction AD and GD in the patients with RA, the purpose of the present study was to investigate AD and GD activities in patients with RA classified into three groups and in regard with their treatment.

Materials and methods

Patients and controls

The study was approved by the Ethics Committee, Medical Faculty, Firat University, Elazığ, Turkey. All participants gave written consent, confirming their acceptance for giving blood through vena brachialis and were informed about the whole experimental procedures. The patients were diagnosed and classified by clinicians of the Internal Medicine Clinic and the control group was selected among healthy parents or siblings.

The study was performed in 32 RA patients (21 male and 11 female) aged 29 to 77 years; mean age 50 years (48 years for men and 52 years for women). None of them had an alcohol abuse problem. The patients had not received any systemic therapy, which might affect cellular immunity during the 3 weeks prior to sample collection. The control group consisted of 52 healthy volunteers precisely matched for age and sex. The women who were included in the study had not been taking oral contraceptives for at least 6 months before sample collection. All patients had active disease according to the criteria of the American Rheumatism Association (Arnett et al. 1988) and the median duration of illness was 7.36 years (range 1-15 years) (Onal et al. 2011).

Preparation of blood samples

Fasting blood samples were collected by standard clinical procedures. EDTA was used to prevent coagulation. Whole blood (1 ml) was used for hematological analysis. Serum (2 ml) was used for detection of C reactive protein, rheumatoid factor and anti-streptolysin-o. The serum used for AD and GD analysis was separated by centrifugation for 10 min at 1000 g at +4°C. The plasma and serum samples were stored at -30 °C. Serum was stored for <3 months pending measurement of the AD and GD activities.

Serum AD assay

The activity of AD in plasma was measured according to the method of Giusti and Galanti (1984). Optical density was measured spectrophotometrically at 265 nm in an assay mixture (final volume, 2 ml) containing 0.025 nM adenosine, 10 nM Tris_/HCl (pH 7.4), 0.15 M sodium chloride, 1.25% glycerol, and 0.02 ml of serum. One unit of activity represents the deamination of 1 mM

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adenosine/min at 37°C temperature and is expressed as international units per litre (IU/l).

**Serum Guanosine deaminase (GD) assay**

GD activity was measured as described. Ammonia formed during 30 min incubation at 37 °C in the presence of guanine in a phosphate buffer (pH:7.4) is estimated colorimetrically using a modified phenol-alkaline hypochlorite procedure (Caraway, 1966).

**Statistical analyses**

All results are expressed as means ± SD. Significant values were established with Mann Whitney U test. The SPSS statistical program (version 9.05 software, SPSS Inc. Chicago, Illinois, USA) was used for statistical treatment of the data. p-values of less than 0.05 were regarded as significant.

**Results**

The mean AD and GD activities in serum of patients with RA are shown in Figures 1 and 2, respectively. The mean values for controls and patients were 18.62 IU/l and 28.4 IU/l for AD and 1.19 IU/l and 1.76 IU/l for GD activity, respectively. GD were significantly (p<0.01) higher but the AD activities were higher (p<0.01) in the controls than in patients. However, serum GDA activities were lower in RA patients than the control subjects (p<0.01). There was no significant difference for both the enzymes among the groups classified in regard with their treatment (p>0.05) (Table 1).

**Discussion**

Immunological system dysregulation, T-cells and B-cell activation, immune-active cell infiltration into the affected regions followed by accelerated neutrophil chemotaxis and phagocytes have been implicated in patients with RA (Klarenbeek et al. 2009). Increased serum AD activities have been observed in many infectious diseases caused by microorganisms infecting mainly the macrophages, in tuberculosis, leprosy, visceral and cutaneous leishmaniasis, brucellosis and, in human deficiency virus (HIV) infection (Valls et al. 1990, Gakis et al. 1991, Erel et al. 1998). It was reported that 95% of serologically positive typhoid fever cases found to have increased ADA activity (Ungerer et al. 1996). RA is a inflammatory disease and the ADA and GA enzyme activity changes may an indicator for detection of the disease. Hence, we measured serum AD and GA activities in patients with RA as an indicator of the disease.

Two ADA isozymes are known as ADA1 and ADA2. While human tissue extracts contained ADA1 predominantly, ADA2 was the main component of serum ADA. Therefore, ADA activity measured in serum reflects ADA2 activity (Fox and Kelley, 1978; Antonioli et al. 2012). In the report of Kobayashi et al. (1993) it was found that the ADA2:ADA ratio decreased in acute hepatitis, but increased in chronic active hepatitis and liver cirrhosis. In early studies on ADA activity in sera of active and chronic liver diseases, it was suggested that monitorizing of AD levels in PBMC may show biochemical and/or histological remission in chronic liver

![Figure 1. Serum AD Activities in Patients with Rheumatoid Arthritis and Healthy Controls (Mean ± SE). *p<0.01 relative to controls.](image)

![Figure 2. Serum GA Activities in Patients with Rheumatoid Arthritis and Healthy Controls (Mean ± SE). *p<0.01 relative to controls.](image)

<table>
<thead>
<tr>
<th>Stages of RA</th>
<th>AD (IU/l)</th>
<th>GD (IU/l)</th>
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</thead>
<tbody>
<tr>
<td>Stage 1 (n=5)</td>
<td>26.58 ± 5.84</td>
<td>2.53 ± 1.27</td>
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<tr>
<td>Stage 2 (n=21)</td>
<td>22.31 ± 1.52</td>
<td>0.95 ± 0.11</td>
</tr>
<tr>
<td>Stage 3 (n=6)</td>
<td>21.32 ± 2.70</td>
<td>0.91 ± 0.10</td>
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</tbody>
</table>
Role of AD and GA activities in serum of patients with Rheumatoid Arthritis

Adenosine deaminase (AD) and guanosine 5’-nucleotidase (GA) activities are well-defined phenomena in patients with RA. These activities are increased in a clinical situation, may be used to assess the severity of an injury. Furthermore, it appears that plasma AD and GA activity can be used for the diagnosis of RA in patients than controls. This was to be expected, since increased cellular and humoral activities as well as the infiltration of T cells into the affected regions followed by a second phase of neutrophil chemotaxis are well-defined phenomena in patients with RA (Katchamart et al. 2010).

In conclusion, this study further supported our previous studies and demonstrated the particular role of AD and GA activities in serum of patients with RA. These changes can provide insights into pathophysiology and, in a clinical situation, may be used to assess the severity of an injury. Furthermore, it appears that plasma AD and GA activity can be used for the diagnosis of RA disease to support clinical findings and as an index for disease. It can also be used during the follow-up period of the patients as well as to monitor the effect of the treatment, if further studies demonstrate its sensitivity or specificity.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

References


