

## Serum ADA and C-reactive Protein in Rheumatoid Arthritis

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**KEYWORDS** Rheumatoid arthritis; adenosine deaminase; cell-mediated immunity; immuno enzyme marker; inflammation; C-reactive protein

**ABSTRACT** Immunological and inflammatory reactions play a pivotal role in the initiation and perpetuation of rheumatoid arthritis. The present study is an attempt to estimate the levels of adenosine deaminase (ADA) activity, a marker for cell mediated immunity and C-reactive protein a marker for inflammation in patients with rheumatoid arthritis. 75 cases presenting rheumatoid arthritis and same number of age and sex matched healthy controls were included in the study. Serum ADA activity was measured spectrophotometrically at 630 nm and serum C-reactive protein was detected using Avitex CRP kit, which is a rapid latex agglutination test. The mean ADA levels were  $59.79 \pm 21.09$  in patients and  $20.71 \pm 5.63$  in controls, significant at  $p < 0.01$ . CRP test was found to be positive in 69/75 cases of RA and none of the controls. The present study observed the importance of ADA as a serum marker in addition to CRP for better therapeutic management of RA.

### INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease characterized by synovial inflammation and subsequent tissue damage. It affects approx 1-2 % of world's population (Deborah et al. 2002). In India alone there are some 10 million people with RA.

It is associated with reduced life expectancy and is a major cause of chronic disability and handicap. Hypertrophy and inflammation of the soft tissues around synovial joints is the common phenomenon occurring in RA. The etiology of RA is not known but it is classified as one of the autoimmune disease (Cassim et al. 2002). There is a prominent immunological dysfunction in the joints and many other tissues by accumulation of chronic inflammatory cells including T and B-lymphocytes, monocytes and macrophages.

Adenosine deaminase (ADA, adenosine amino hydrolase E.C. 3.5.4.4) is an enzyme involved in the metabolism of purine bases, catalyzing the deamination of adenosine, forming inosine in the process (Fox and Kelly 1998). It's main physiological activity is related to lymphocytic proliferation and differentiation. As a marker of cell mediated immunity, its activity is

found to be elevated in those diseases in which there is a cell – mediated immune response (Galanti et al. 1981; Piras et al. 1982).

CRP an acute phase protein is synthesized by hepatocytes in response to proinflammatory cytokines in particular IL-6. It has been shown to be of great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA (Molenaar et al. 2001).

Therefore the present study is an attempt to estimate the levels of adenosine deaminase (ADA) a marker for cell mediated immunity and C-reactive protein a marker for inflammation in Indian patients with RA.

### MATERIALS AND METHODS

75 patients presenting rheumatoid arthritis attending Sri Deepti Rheumatology Centre, Hyderabad were included in the study. The diagnosis of RA was established by clinical analysis, ESR and rheumatoid factor tests. Equal number of age and sex matched healthy individuals with no known history of any disease were taken as controls. All the subjects were examined clinically and information pertaining to age, sex, habits and health status was recorded in special case proforma. Blood samples were collected from both controls and patients for the estimation of serum ADA and C-reactive protein.

**ADA Estimation:** The serum was assayed immediately for ADA activity at 37° C by a

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spectrophotometric method using adenosine as the substrate. This method is based on the Bertholet reaction, that is, the formation of coloured indophenol complexes from ammonia liberated from adenosine and quantitated spectrophotometrically at 630 nm (Guisti and Galanti 1984). One unit of ADA is defined as the amount of enzyme required to release one micromole ammonia per minute from adenosine at standard assay conditions. The activity of ADA is expressed in units / litre.

**C-Reactive Protein Detection:** For the detection of CRP in serum, Avitex -CRP kit was used which is a rapid latex agglutination test. The test is based on the principle that Avitex-CRP latex particles are coated with antibodies to human CRP, i.e. when the latex suspension is mixed with serum containing elevated CRP levels on a slide; clear agglutination is seen within 2 minutes. Avitex -CRP has detection limit of 6 mg/ litre of CRP in the patient's serum. The test is considered as positive when the CRP serum concentration is above 6mg/litre and negative when it is at 6 mg / litre and below.

**Statistical Analysis:** The data of the study subjected to statistical analysis is expressed as mean  $\pm$  SD. Statistical comparisons were performed by Student 't' test.

## RESULTS

Detailed clinical examinations were performed by the rheumatologist of the centre and 75 patients with confirmed diagnosis of rheumatoid arthritis were included in the study. Adenosine deaminase levels and C- reactive protein were determined and are presented in tables 1 to 4.

**Age and Sex:** In the present study patients of rheumatoid arthritis belonged to the age group of 20-60 years. Among 75 patients of RA 18 patients were males and 57 were females as shown in table 1. The mean  $\pm$  SD of age in males was  $44.35 \pm 13.70$  (yrs) and females was  $44.1 \pm 14.37$  (yrs) and mean age of control males was  $40.46 \pm 8.45$  (yrs) and control females was  $44.64 \pm 12.96$  (yrs).

**Serum ADA Levels:** Mean ADA levels estimated in rheumatoid arthritis patients and controls are presented in table 2. The mean  $\pm$  SD of ADA levels in serum of RA patients was found to be  $59.79 \pm 21.09$  and that of the controls was  $20.71 \pm 5.63$ . The difference in the mean values was statistically significant at  $p < 0.01$ .

**Serum CRP Levels:** CRP levels estimated in the RA patients and controls are presented in tables 3 & 4. In the present study 69/75 cases of RA were found to be positive to CRP while all the controls were negative for the test. Serum dilutions were performed to detect the concentration of CRP in all positive cases.

**Table 1: Sex and mean age of Rheumatoid patients**

| Cases of Rheumatoid arthritis (n=75) |                   |
|--------------------------------------|-------------------|
| Sex (male/female)                    | 18/57             |
| Age (mean $\pm$ SD yrs)              |                   |
| Males                                | $44.35 \pm 13.70$ |
| Females                              | $44.1 \pm 14.27$  |

**Table 2: Serum Adenosine deaminase (ADA) levels (Units/lit) in patients with Rheumatoid Arthritis and Controls**

| Groups      | Number of cases | Serum ADA Mean $\pm$ SD |
|-------------|-----------------|-------------------------|
| RA patients | 75              | $59.79 \pm 21.09^*$     |
| Controls    | 75              | $20.71 \pm 5.63$        |

Significant at  $p < 0.01$

**Table 3: Serum CRP levels (mg/liter) of patients with Rheumatoid Arthritis and Controls**

| Groups      | No. of cases | No. of cases positive for CRP |
|-------------|--------------|-------------------------------|
| RA Patients | 75           | 69                            |
| Controls    | 75           | NIL                           |

**Table 4: Semi -Quantitative analysis of CRP levels (mg/litre) of patients with Rheumatoid Arthritis**

| Dilution | +ve for CRP N=69 | Concentration of CRP |
|----------|------------------|----------------------|
| 1: 2     | 22               | $2 \times 6 = 12$    |
| 1: 4     | 19               | $4 \times 6 = 24$    |
| 1: 8     | 16               | $8 \times 6 = 48$    |
| 1:16     | 12               | $16 \times 6 = 96$   |

## DISCUSSION

The disease activity in RA is an expression of a cascade of immunological and inflammatory reactions, probably initiated by an unknown stimulus.

Recent evidence in various fields has consistently indicated that T -cells play a key role in initiating and perpetuating the inflammation. The prominence of T-cells and monocyte/macrophages in rheumatoid synovium suggests that T cells may localize and amplify the effector functions of monocyte/macrophages in rheumatoid disease (Stamp et al. 2004).

T cells activated by dendritic cells or infla-

mmatory cytokines, in turn activate monocytes/macrophages, endothelial cells, smooth muscle cells and fibroblasts to produce proinflammatory cytokines (tumour necrosis factor alpha, interleukin-6), chemokines, tissue factor, the main inhibitor of the coagulation cascade *in vivo* and finally matrix metalloproteinases responsible for tissue destruction (Monaco et al. 2004).

ADA has been considered as a marker for cell mediated immunity. Its role in cellular immune function was known following the detection of reduced levels in patients with severe combined immunodeficiency (Vander weyden and Kelley 1977). It has strongly been suggested that serum ADA activity reflects monocyte/ macrophage activity or turnover in different diseases. (Gakis et al. 1989; Ungerer et al. 1992).

Increased serum ADA activities have been observed in many infectious diseases caused by microorganisms such as tuberculosis, leprosy, brucellosis and in human immunodeficiency virus (HIV) infection. Gakis et al. (1991) observed high ADA activities in the sera of patients with visceral leishmaniasis (Kalaazar).

Serum Adenosine deaminase activity was also found to be high in myocardial infarction (Jyothy et al. 2003) and unstable angina (Surekha et al. 2003) suggesting the contribution of immunological and inflammatory process in the pathogenesis of coronary heart disease.

The results of the present study in rheumatoid arthritis patients indicated highly significant mean levels of ADA in patients compared to controls. Hitoglou et al. (2001) has also investigated the activity of total ADA (t ADA) in serum of juvenile RA and systemic lupus erythematosus in different phases of the disease and showed that increased ADA activity correlated closely with clinical disease activity and relapse.

In another report Sari et al. (2003) investigated the correlation between the activity of total ADA and isoenzymes ADA1 and ADA2 and clinical activity in patients with rheumatoid arthritis and concluded that serum t ADA and ADA2 activity is closely associated with RA and further suggested that these non-invasive investigations can be used as biochemical markers for inflammation which may provide additional information regarding disease activity along with the traditional indices such as ESR and CRP.

Inflammatory processes play a pivotal role in the pathogenesis of rheumatoid arthritis. The

prototypic marker of inflammation is C-reactive protein (CRP) a member of the pentraxin family. The production of CRP in the liver is triggered by various proinflammatory cytokines derived either from monocytes and or macrophages. The proinflammatory response results in the increased secretion of interleukin -1  $\beta$  and tumor necrosis factor -  $\alpha$  which then results in the release of the messenger cytokine, interleukin - 6 which stimulates the liver to secrete CRP. It was thought as a bystander marker of inflammation, without playing a direct role in the inflammatory process.

Recent studies suggest that CRP may also contribute directly to the proinflammatory state. CRP stimulates monocyte release of inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and may also directly act as a proinflammatory stimulus to phagocytic cells. (Ballore et al.1992, Bharadways et al. 1999, Stankcikova and Rovensky 1993).

In the present study the levels of C-reactive protein were significantly high in the patients compared to controls. Similarly Milovanoic et al. (2004) and Klimiuk et al. (2003) also observed high values of CRP indicative of active inflammation in RA patients. Yildirim et al. (2004) studied the association between acute phase reactant (APR) levels and disease activity score (DAS28) in patients with rheumatoid arthritis and found that serum CRP, among the various APR tests (erythrocyte sedimentation rate (ESR), haptoglobin (Hp), ferritin, and plasma fibrinogen.), is the most useful biochemical marker for evaluating the disease activity of patients with RA.

The understanding of the pathophysiology of RA and precise knowledge of the possible triggers of the inflammation may open novel therapeutic approaches. Hence, the present study suggests the importance of measuring the biomarkers of inflammation assessed in the study not only to determine the severity of inflammation but also to evolve targeted treatment strategies for better management of the condition.

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