

Original Research Article**EVALUATION OF BIOCHEMICAL MARKER FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS*****S.S.Haque, Santosh Kumar, Rekha Kumari, U. Kumar, A Saran, Md.Tanweeruddin**

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ABSTRACT

Objectives: Rheumatoid arthritis (RA) is a chronic auto-immune disorder; 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 macrophage, which is due to result of cell mediated immune response in RA patients. Adenosine deaminase (ADA) is one of the marker of cellular immunity and it is a key enzyme of purine metabolism, play an important role in the determination of the seriousness of an inflammatory process. The aim of this study was to investigate the role of adenosine deaminase in addition to C-reactive protein (CRP) for the diagnosis and therapeutic management of RA.

Material and methods: Catalytic activities of ADA in serum were determined by a spectrophotometric method at 630 nm and serum C-reactive protein detected using Avitex CRP kit, which is a rapid latex agglutination test.

Results: The results showed a statistically significant ADA levels in serum of patients with rheumatoid arthritis ($p < 0.001$). CRP test was found to be positive in 36/40 cases of RA and none of the controls.

Conclusion: ADA assay can be a reliable, sensitive and specific test, and CRP is an important inflammatory marker for rapid diagnosis of rheumatoid arthritis.

Key words: Adenosine deaminase, C-reactive protein, rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is one of inflammatory autoimmune disorder characterized by chronic proliferative synovitis that leads to ultimately bone destruction [1], RA is most common disease of connective tissue which is reported in 2-3% of the world population [2]. In India alone there are more than 10 million people with RA. Etiology of RA, and most of the rheumatic diseases, is still to a great extent unknown and unclear. A problem in diagnosing occurs because of the most often present typical and gradual start of RA with unclear and uncharacteristic clinic signs and asymptomatic intervals [3]. In clinical practice, RA is diagnosed respecting revised criteria of ACR (American College of Rheumatology) from 1987 and must last at least for 6 weeks [3]. ACR criteria for RA are mostly used as a "golden standard" in RA diagnosing although they have a limit for early diagnosing of RA, having in mind that they mainly comprise manifest clinical symptoms which are not necessarily shown in early stage of the disease [3]. At the same time, those are the reasons why it is necessary to find a new "golden standard" that would be less dependent on RA clinical symptoms [3].

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 [4]. Its 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 [5, 6]. During inflammatory process, this enzyme is released in extra cellular and serosal fluids and produces different levels of ADA. The levels depend on the numbers of nuclear cells, especially T cells and macrophages [7].

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 [8].

For a long time, the diagnosis of RA was mainly based on clinical manifestations. However, it is often difficult to diagnose RA in the very early phases of the disease and in many cases irreversible damage had occurred by the time the diagnosis was confirmed. Therefore, laboratory tests which are sensitive and specific early in the disease course are desirable to allow earlier diagnosis and intervention. A multitude of such biomarkers have been investigated focusing on analytes found in the different cellular compartments including biomarkers involved in the synthesis and degradation of bone/cartilage, inflammation or autoimmune processes in order to identify those that could be clinically useful.

So in the light of the above statement my aim of the study is to investigate the role of ADA level along with CRP for the diagnosis of RA.

MATERIALS AND METHODS

Patients: The study involved 80 subjects who were divided into three groups. The control group consisted of 40 healthy subjects (26 women and 14 men) with an average age of 55.38 years, who were from 30 to 70 years old; they also did not have family history of rheumatoid arthritis and they were not medically treated. From the remaining 40 subjects were diagnosed with rheumatoid arthritis. All the subjects were diagnosed with RA by specialized rheumatologists. Criteria for involving the patients in the study and for their exclusion were the revised ACR criteria from 1987.

Blood samples were collected from eighty patients who were attending to Indira Gandhi Institute of Medical Sciences Patna teaching hospital from February to September. Sera were separated and stored at -20 °C until use.

Methods

Catalytic activity of ADA was determined by Giusti's modified spectrophotometric method [9] using adenosine (Sigma, Aldrich) as a substrate, and the results were read by spectrophotometer. The method principle was the following: by adenosine deaminase reacting, ammonium is released from adenosine and together with phenol nitroprusside reagent and sodium hypochlorite alkaline solution gives indophenol blue colour whose intensity is proportional to the amount of released ammonium that is the catalytic activity of total adenosine deaminase, which is measured at 620 nm. The activity of ADA is expressed in units / litre. Reference Range of ADA catalytic activities for this method was 13.20-20.80 U/L.

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's 't' test.

RESULTS:

Form 40 patients, 26 (65%) of them were women while only 14 (35%) of who were men. The mean age of the patients was 55.38 ± 10.05 (55.26 ± 7.93 for women and 54.85 ± 8.23 for men).

A mean ADA value in serums of the control group was $X = 17.57 \pm 2.07$ U/L. As shown in table 1 results indicated that there is a significant differences between RA patients ($37.12 \pm 8.02^*$ U/L) and control group (17.57 ± 2.07 U/L).

CRP levels estimated in the RA patients and controls are presented in table 3 and 4. In the present study 36/40 cases of RA were found to be positive to CRP while all of controls was negative for the test. Serum dilutions were performed to detect the titer of CRP in all positive cases.

Table 1. Sex and mean age of rheumatoid patients

Groups	Number of subjects(N)	Mean \pm SD
Age		
1. Male	14	54.85 ± 8.23
2. Female	26	55.26 ± 7.93

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

ADA in serum	Number of subjects(N)	Mean \pm SD
1. RA patients	40	$37.12 \pm 8.02^*$
2. Controls	40	17.57 ± 2.07

Significant at $*p < 0.001$

Table -3: Serum C-reactive protein in patients with rheumatoid arthritis and controls

Groups	No. of cases	No of cases positive for CRP
Rheumatoid arthritis	40	36
Controls	40	Nil

Table-4: Semi quantitative analysis of C-reactive protein levels of patients with rheumatoid arthritis

Dilution	+ ve for CRP No =36	Titer of CRP
1:2	16	12
1:8	10	24
1:16	8	48
1:32	2	96

In this study, significant difference was found between the mean value of serum ADA among RA patients when compared with the control group (table 2) and this may be because increase activity of ADA is caused by its releasing from damaged cells.

Discussion:

ADA is one of the most essential immune enzymes and plays a critical role in proper development of the T and B-lymphocytes in mammals [10, 11]. In the disease process of RA the immunological and inflammatory reaction play an important role. The T-cells activated by

dendritic cells or inflammatory cytokines molecules, which in turn produces monocytes/macrophages, endothelial cells, smooth muscle cells and fibroblasts to produce proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α), and interleukin-6 (IL-6), the main inhibitor of the coagulation cascade *in vivo* and finally matrix metalloproteinase's responsible for tissue destruction [12] in RA diseases.

It is still not completely clear what exactly causes the increase in ADA catalytic activities in the serum of patients suffering from RA, but it is supposed that ADA catalytic activity is increased because of its release from the damaged cells and the increased cell proliferation in RA [13].

C-reactive protein (CRP) is one of the most responsive acute-phase serum reactants produced by liver. CRP produces various proinflammatory cytokines derived either from monocyte and/or macrophages, and it reflects more short-term changes in disease activity associated with joint destruction. In addition, CRP determination is widely available, easy to perform and of low cost, making it the preferred biomarker of disease activity and play a pivotal role in pathogenesis of rheumatoid arthritis. In the present study the levels of C-reactive protein were significantly high in patients compared to controls and high values of CRP indicates of active inflammation in RA patients.

CONCLUSIONS:

During recent years, a huge number of potential biomarkers for the diagnosis RA have been investigated but only ADA and CRP are reliable, cost-effective and currently tested routinely in clinical practice. During inflammations of RA, ADA is released in extra cellular location, resulting in the considerable increase of its activity by activating CMI, which ultimately help to better understanding of some pathophysiological aspects of a disease and may help to relieve the triggering factors of inflammation and promote new therapeutic approaches.

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