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Original Research Article

A Study of Serum Adenosine Deaminase Activity in Patients with Rheumatoid Arthritis

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Abstract

Background: Numerous studies suggest that serum adenosine deaminase (ADA) activity could serve as a potential diagnostic marker for rheumatoid arthritis (RA). However, no independent study has validated this finding in our population. This study aims to measure total ADA activity in the serum of our RA patients and assess its diagnostic potential.

Methods: The study included 50 RA patients who sought medical care at Prathima Institute of Medical Sciences, Karimnagar. An equal number of age- and sex-matched healthy controls were also enrolled. Blood samples were collected from all participants and analyzed for serum total ADA activity, C-reactive protein (CRP), and rheumatoid factor (RF).

Results: Serum total ADA activity was significantly higher ($p<0.01$) in RA patients (30.9 ± 10.6 U/L) compared to healthy controls (13.66 ± 3.75 U/L). However, no significant difference ($p>0.05$) in ADA activity was observed between smokers and non-smokers within the RA group. Among the 50 RA patients, only 13 (26%) tested positive for CRP, and 11 (22%) tested positive for RF.

Conclusion The significant difference in ADA activity between RA patients and healthy controls highlights its potential utility as a marker in diagnosing the disease within our population, especially when considered alongside clinical background.

Keywords: Rheumatoid arthritis, Adenosine deaminase, C-reactive protein, Rheumatoid factor.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that, if left uncontrolled, can lead to joint damage, disability, a reduced quality of life, and an increased risk of cardiovascular and other co-morbidities. The disease affects approximately 0.5–1.5% of the adult population in industrialized nations, with a higher prevalence among women and the elderly [1]. Although the exact cause of RA remains unknown, genetic predisposition, obesity, and smoking are recognized as major risk factors [2, 3]. Currently, the diagnosis of RA is based on the American Rheumatism Association's (ARA) revised criteria, radiographic evidence, and routine blood tests such as ESR, CRP, and RF [4, 5]. Early and accurate diagnosis is crucial for initiating timely clinical interventions to prevent irreversible joint destruction, which could progress to erosive, destructive, and disabling forms [6, 7]. While routine blood tests provide some evidence supporting radiographic and clinical findings, their sensitivity and specificity are often inadequate, making them insufficient as sole diagnostic tools [8]. Consequently, there is an ongoing need for more sensitive and specific diagnostic markers that are reliable, simple, and rapid to detect. Adenosine deaminase (ADA, EC 3.5.4.4), an enzyme involved in the purine metabolic pathway, has emerged as a potential diagnostic marker for RA. This enzyme catalyzes the deamination of adenosine to inosine and deoxyadenosine to deoxyinosine in mammalian cells [9]. ADA is primarily present in serum and lymphoid tissues and plays a crucial role in the maturation and function of T lymphocytes and macrophages [10, 11]. It exists in two isoenzyme forms, ADA1 and ADA2, which are encoded by separate genes [12]. ADA activity significantly increases during inflammatory conditions due to an elevated number of nucleated cells, particularly T lymphocytes and macrophages. Since RA is a chronic inflammatory disease, it is reasonable to expect an increase in serum ADA activity. Several studies have supported this hypothesis, demonstrating elevated serum ADA activity in RA patients [13]. However, no study has yet explored

the potential of ADA as a diagnostic marker for RA among our population. This study aims to investigate whether serum ADA activity is elevated in patients of RA patients and evaluate its potential as a diagnostic tool for routine clinical use.

Materials and Methods

This cross-sectional study was conducted in the Department of General Medicine, Prathima Institute of Medical Sciences, Naganoor, Karimnagar. Institutional Ethical approval was obtained for the study. Written consent was obtained from all the participants of the study after explaining the nature of the study in the vernacular language.

A total of 50 newly diagnosed RA patients who had not yet received any medication, along with an equal number of age- and sex-matched healthy controls, were included in this study. Informed consent was obtained from all participants before their enrollment. The diagnosis of RA in the patients was confirmed by a rheumatologist based on the ARA revised criteria, radiographic findings, and results of ESR, CRP, and RF tests. Patients with co-existing conditions such as tuberculosis, diabetes mellitus, cardiovascular diseases, HIV/AIDS, or other musculoskeletal disorders (e.g., osteoarthritis, osteoporosis, spinal disorders, severe limb trauma, or gouty arthritis) were excluded from the study. Relevant details, including name, age, sex, hospital number, contact information, family history of RA, and smoking habits, were recorded for all participants using a structured case proforma. Samples from all subjects were collected in sterile vials and allowed to clot at room temperature. The serum was carefully separated from the clotted blood and either stored at -20°C for later analysis or analyzed immediately for total ADA activity, CRP, and RF at the clinical biochemistry laboratory.

Measurement of Serum Total ADA Activity

Serum total ADA activity was measured using a commercially available kit, following the manufacturer's protocol (Tulip Diagnostics (P) Ltd, Verna Goa, India). The assay employed a colorimetric method described by Galanti and Guisti (1984). One unit of ADA activity was defined as the amount of enzyme needed to release three micromoles of ammonia per minute from adenosine within one hour at 37°C. The results were expressed in international units per liter (U/L).

Detection of CRP

The presence of elevated CRP levels in serum was determined using a rapid latex agglutination test with a commercially supplied CRP-Latex kit (Span Diagnostics Ltd, Surat, India). This test relies on latex particles coated with antibodies against human CRP. When mixed with serum containing elevated CRP levels, visible agglutination occurred within two minutes. The kit had a detection limit of 6 mg/L for CRP. A result was considered positive when CRP levels exceeded 6 mg/L and negative when the levels were 6 mg/L or below.

Detection of RF

Serum RF was measured using a commercially available RF latex reagent kit (RFCL Limited, Uttarakhand, India), following the manufacturer's instructions. Similar to the CRP test, the RF test was based on the principle of latex agglutination. The test's sensitivity was 10 IU/L, and agglutination observed within two minutes was considered a positive result.

Statistical Analysis

Data analysis was conducted using SPSS version 22. Tables and graphs were created with SPSS and Microsoft Excel. Descriptive statistics, including frequencies, standard deviations, and percentages, were calculated for all variables. Categorical data were analyzed using the Chi-square test for statistical significance. A two-tailed p-value of <0.05 was considered statistically significant.

Results

The RA patients enrolled in this study exhibited a diverse range of clinical signs and symptoms. Among them, 42 patients (84%) reported bilateral joint pain as their primary complaint, while 8 patients (16%) experienced unilateral joint pain. Morning stiffness lasting approximately two hours was observed in 35 patients (70%), whereas the remaining 15 patients (30%) did not report this symptom. Joint pain was localized to the wrists, metacarpals, and interphalangeal joints in 15 patients (30%), while 25 patients (50%) experienced pain in the knees and ankles.

The mean age of the RA patient group was 47.55 ± 10.9 years, comprising 15 males and 35 females. The control group, in comparison, had a mean age of 48.02 ± 11.37 years and included 20 males and 30 females. Among the RA patients, 10 individuals (20%) were smokers, while 40 (80%) were non-smokers.

Of the 50 RA patients, 13 (26%) tested positive for CRP, and 11 (22%) tested positive for RF. The mean total ADA activity in RA patients was significantly higher (30.9 ± 10.6 U/L) compared to the healthy control group (13.66 ± 3.75 U/L), with a statistical significance of $p<0.001$. However, no statistically significant difference ($p>0.05$) in ADA activity was observed between smokers (31.0 ± 12.0 U/L) and non-smokers (29.0 ± 8.2 U/L) within the RA patient group (Table 1).

Table 1: Status of serum CRP, RF, and total serum activity of ADA in cases of rheumatoid arthritis (50 cases) included in the study

	Sex	Mean age in years	CRP +ve	RF +ve	Total ADA activity levels in U/L	Mean ADA activity U/L	P value
RA patients (50)	Male (15)	47.55 ± 10.9	7	6	31.21 ± 10.9	30.9 ± 10.6	0.001*
	Female (35)		6	5	28.92 ± 9.2		
Controls (50)	Male (20)	48.02 ± 11.37	0	0	12.68 ± 3.3	13.66 ± 3.75	
	Female (30)		0	0	13.1 ± 3.6		

Discussion

RA disproportionately affects women, with women being approximately twice as likely to develop the condition compared to men [14]. Additionally, 80% of individuals with RA experience onset between the ages of 35 and 50 years [15]. Our study found that women were affected 1.8 times more frequently than men, aligning closely with previously reported data. The mean age of patients in our study was 47.55 ± 10.9 years, consistent with earlier findings [16]. Current research on RA predominantly focuses on its immunological aspects, driven by the inflammatory response responsible for joint and tissue damage. The T-lymphocyte-mediated nature of the disease, characterized by T-cell infiltration into affected joints and the subsequent recruitment of macrophages and fibroblasts, is well-established [17]. However, certain diagnostic markers have shown limitations. For instance, antinuclear antibodies (ANA), present in 30-60% of RA patients, lack specificity as they are also associated with other conditions such as systemic lupus erythematosus and scleroderma [18]. Similarly, rheumatoid factor (RF), which is detected in 72-85% of adult RA patients—particularly in high titers—tends to indicate advanced and destructive stages of the disease, reducing its utility as a reliable diagnostic marker [19]. CRP has also been found to have limited predictive value for RA onset [20]. In our study, only 16 out of 69 RA patients tested positive for CRP, and 11 were positive for RF (Table 1), highlighting the restricted diagnostic reliability of these markers.

A more promising candidate for RA diagnosis is adenosine deaminase (ADA). Elevated serum ADA activity, including its isozymes ADA1 and ADA2, has been shown to correlate with RA clinical activity [13]. Additionally, ADA activity in both serum and synovial fluid has demonstrated utility in differentiating RA from conditions like osteoarthritis and reactive arthritis [21]. In this study, the mean total ADA activity in RA patients was 30.9 ± 10.6 U/L, which is lower than the 59 U/L reported by Sari et al. [22] Similarly, the mean ADA activity in our healthy controls (13.66 ± 3.75 U/L) was lower than their reported value of 20.71 ± 5.63. These discrepancies could arise from differences in estimation methods or reflect genuine population variations, underscoring the need for further research to define ADA activity ranges across diverse populations. This study revealed a moderate positive correlation between serum ADA activity and RA disease activity scores (r = +0.462), suggesting ADA's potential as a biochemical marker for RA when considered alongside traditional indices such as CRP. Furthermore, while smoking is a known etiological factor for RA, our findings indicate it may play a role in initiating rather than progressing the disease, as no statistically significant difference in serum ADA levels was observed between smoking and non-smoking RA patients.

Conclusion

The significant difference in ADA activity between RA patients and healthy controls highlights its potential utility as a marker in diagnosing the disease within our population, especially when considered alongside clinical background data. However, it is important to note that this is a hospital-based study conducted in South India and may not represent the entire Indian population. To generalize the applicability of serum ADA for the early diagnosis of RA, a more comprehensive study involving a representative population from various regions of the country is required.

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