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COMPARISON OF ADENOSINE DEAMINASE ACTIVITY, SERUM C-REACTIVE PROTEIN AND RHEUMATOID FACTOR IN MID AND FAR WESTERN NEPALESE PATIENTS WITH RHEUMATOID ARTHRITIS

*** Priti SINGH, **Salman KHAN *Rabindra Kumar MITTAL**

*Department of Biochemistry, **Department of Microbiology, Nepalgunj Medical College and Teaching Hospital Banke, Nepal

Correspondence Author: Priti Singh: Email: priti186631@gmail.com

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*Department of Biochemistry, **Department of Microbiology, Nepalgunj Medical College and Teaching Hospital Banke, Nepal

Correspondence Author: Priti Singh: Email: priti186631@gmail.com

ABSTRACT:

Adenosine deaminase (ADA) is introduced as helpful marker in diagnosis, prognosis and monitoring of treatment in rheumatoid arthritis (RA). The aim of this study was to investigate serum ADA activity in Nepalese patients with RA. The objective was to assess the diagnostic potential of serum ADA activity for routine diagnosis of RA. This was a Hospital based case-control study conducted between March 2012 and February 2013. A total of 58 diagnosed patients of RA and 58healthy controls were included in this study after obtaining their informed consent. All the patients fulfilled the criteria of the American rheumatism association. Blood samples were collected, analyzed for serum total ADA, C-reactive protein (CRP) and rheumatoid factor (RF). The serum total ADA activity was found to be significantly ($p<0.0001$) higher (34.10 ± 8.02 U/L) in all RA patients compared to healthy controls (15.21 ± 4.40 U/L). Among the 58 patients with RA,15 (25.9%)had elevated for CRP and 10 (17.24 %) were positive for RF test. Results showed, ADA catalytic activity in serum can be a useful biochemical marker for the diagnosis of RA in the Nepalese population with relevant clinical scenarios when there is absence of CRP and RF in the serum.

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

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INTRODUCTION:

Rheumatoid arthritis (RA) is a systemic inflammatory disease which usually affects the joints of hands and feet as an erosive, symmetrical polyarthritis. Although the cause of Rheumatoid arthritis is unknown, it is considered as an autoimmune disorder involving other joints and organs [1]. The worldwide prevalence of RA was reported as approximately 1.0%, while it is more common in 7th decade of the life (60-69 age) [2]. One of the potential diagnostic markers that is actively being pursued for RA is Adenosine deaminase, an enzyme, which is present in red cells and the vessel wall catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine. Inosine and 2'-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid [3]. The enzyme exists in two isoenzyme forms: ADA1 and ADA2, coded for by separate genes [4].ADA is considered as a good marker of cell mediated immunity [5]. High lymphocyte ADA activities were found to be elevated in diseases in which there is cell mediated immune response [6].CRP an acute phase protein is synthesizedby hepatocytes in response to pro-inflammatorycytokines in particular IL-6. CRP has been shown tobe of great value as an inflammatory marker inRA and has been suggested to mediate part ofthe complement activation in RA [7].

As yet there has not been such study except one (In UCMS Bhairwa) which demonstrates the suitability of ADA as a potential diagnostic marker for RA in Nepalese population. Therefore the aim of this study was to investigate serum ADA activity in Nepalese patients with RA. The objective was to assess the diagnostic potential of serum ADA activity for routine diagnosis of RA

SUBJECTS AND METHODS:

This was a hospital based case-control study conducted at the Nepalgunj Medical College and Teaching Hospital, Banke, Nepal between March 2012 and February 2013. A total of58 diagnosed patients of RA without any medication and 58 (age and sex matched) healthy controls were selected for this study. Informed consent was obtained from each subject. All the patients included in this study fulfilled the criteria of the American rheumatism association (ARA)[8]. RA patients with tuberculosis, diabetes mellitus, cardiovascular diseases, HIV/AIDS and patients with other types of musculoskeletal disorders, osteoarthritis, osteoporosis, spinal disorders, severe limb trauma and gouty arthritis were excluded from the study. Ethical approval for the study was obtained from the institutional research ethical committee.

From each patient and control 5.0 ml of venous blood was collected in a sterile vial and allowed

to clot at room temperature. The sera were carefully separated from the clotted blot and either stored at -20°C for later analysis or analyzed immediately for total ADA activity, CRP and RF at the central laboratory of biochemistry of the Nepalgunj medical college. The total activity of serum ADA was assayed with a commercially supplied kit (Tulip Diagnostic (P) Ltd, Verna Goa, India) according to the protocol of the manufacturer. The assay was based on the colorimetric method described by Galanti and Guisti [9]. Reference interval of total ADA catalytic activities for using this method was 13.20–20.80 U/L [9]. One unit of ADA was defined as the amount of enzyme required to release three micromoles of ammonia per minute from adenosine in one hour at 37°C [9].

The presence of elevated CRP level in the serum was detected by a rapid latex agglutination test using a commercially supplied CRP-Latex kit (RFCL Limited, Uttarakhand, India). The test is based on the principle that CRP-Latex particles are coated with antibodies to human CRP and when the latex suspension is mixed with serum containing elevated CRP levels on a slide; clear agglutination is seen within 2 minutes. CRP-Latex had detection limit of 6.0 mg/L of CRP in serum. The test was considered positive when the CRP concentration was 6.0 mg/L or greater and negative when it was below than 6.0 mg/L. [10]

Serum RF was detected by using a commercially supplied RF latex reagent kit (RFCL Limited, Uttarakhand, India). The test was performed as per the protocol of the manufacturer. It is based on the principle of rapid latex agglutination slide test similar to the one described above for CRP. The sensitivity of this latex test was 10.0 IU/ml of RF. The test was considered positive if the agglutination was observed within two minutes. [11]

The results obtained from the above investigation were analyzed and expressed as mean ± SD by using Excel 2007 data pack. The statistical comparison was done by student t-test using SPSS version 16, Chicago, USA.

RESULTS:

In the present study patients with rheumatoid arthritis and the non-rheumatoid arthritis controls were in the age range of 20-60 years. Among the 58 patients with RA 20 (34.5%) were males and 38 (65.5%) were females as shown in Table 1. The mean ± SD of age of the males and females was 46 ± 13.08 years and in controls was 45 ± 14.01 years.

Table-1: Gender distribution and mean age of the Non rheumatoid arthritis controls and Rheumatoid arthritis patients

Groups	Males	Females	Mean age (years)
Non rheumatoid arthritis controls (n = 58)	29 (50.0%)	29 (50.0%)	45±14.01
Rheumatoid arthritis patients (n = 58)	20 (34.5%)	38 (65.5%)	46±13.08

Table-2: Serum CRP levels (mg/liter) of patients with Rheumatoid Arthritis and Controls

Groups	Gender distribution		Elevated serum CRP
Non rheumatoid arthritis controls	Males	29	0
	Females	29	0
Rheumatoid arthritis patients	Males	20	9 (45.0%)
	Females	38	6 (15.8%)

Table 3: RF test of patients with Rheumatoid Arthritis and Controls

Groups	Gender distribution		RF test (positive)
Non rheumatoid arthritis controls	Males	29	0
	Females	29	0
Rheumatoid arthritis patients	Males	20	6 (30.0%)
	Females	38	4 (10.53%)

Table 4: Serum Adenosine deaminase (ADA) levels (U/L) in patients with Rheumatoid Arthritis and Controls

Parameters	Non rheumatoid arthritis controls (n=58)	Rheumatoid arthritis patients (n=58)	P value
ADA(U/L)	15.21 ± 4.40	34.10±8.02	<0.0001

Our results show that among the 58 RA patients, only 15 (25.9%)had elevated serum CRP level and 10 (17.2%) had positive RF test (Tables 2 & 3).The results clearly indicate the limitation and inadequacy of using serum CRP level and the RF test parameters for the diagnosis of RA.

The mean total ADA activity for all the RA patients was significantly ($p<0.0001$) higher (34.10±8.02 U/L) as compared to that of the healthy controls (15.21 ± 4.40 U/L). (Table 4)

DISCUSSION:

RA is the most common inflammatory arthritis, affecting about 1.0% of the general population worldwide [12]. Pathogenesis of RA is still not fully understood, there is evidence that CD4+ T cells play a central role in initiating, perpetuating and precipitating chronic inflammation in synovial tissue [13, 14]. Another role of activated CD4+T cells is stimulation of B cells to differentiate into plasma cells producing RF (Rheumatoid Factor) and other auto antibodies [15,16]. ADA is one of the most essential immune enzymes. Its function gives a clear picture of the immune status of the body. [16,17]. A close correlation has been found between the severity of inflammation and local increase in both expression and activity of ADA [18]. ADA plays a crucial role in lymphocyte proliferation and differentiation [19] and shows its highest activity in T- lymphocytes [20].The high plasma ADA activity might be due to abnormal T-lymphocyte responses or proliferation [19].The increased serum level of ADA is indicator of stimulation of cellular immunity. For instance, this condition can be seen in lymphoblastic leukemia, acute hepatitis, human immunodeficiency (HIV) virus infection, infectious mononucleosis, tuberculosis, pneumonia and rheumatoid arthritis. Some have suggested that the major source of the prevalent form of ADA (ADA2) in serum is the monocyte/macrophage cell system [21].The increase in serum ADA level in RA patients can be explained by immunity status changes. In this case, the ADA level reflects the monocyte/macrophage activity or turnover [22]. Many studies demonstrated the elevated serum level of ADA in RA patients [23-25].Rheumatoid arthritis is more common in female as compare to male [26]. RA is likely to affect females approximately two times more than males[26]and 80% of people with RA develop signs and symptoms of the disease between 35 and 50 years of age [27]. Our current study showed that almost 1.9 times more females (65.5%) are affected than males (34.5%) in Nepalese population which is closely similar to the results reported by N. Gautam et al .[28]. Our result indicated that among the 58 RA patients, only 15 (25.9%) have elevated serum CRP and 10 (17.24 %) tested positive for RF. The result obtained in our present study illustrates clearly the limitation in using serum

CRP and RF as parameters for the diagnosis of RA. Our result is closely related with the N. Gautam et al [26], study conducted at the Universal College of Medical Sciences Teaching Hospital, Bhairahawa, Nepal. According to these authors out of 69 RA patients, only 16 (23.1%) have elevated serum CRP level and 11 (15.9%) tested positive for RF.

CONCLUSION:

There was a significant difference in the levels of ADA activity between the RA patients and healthy controls, which may indicate its usefulness in diagnosing the disease in the Nepalese populationwhen taken in the context of clinical background data.

However, this is a hospital based study confined only to mid and far western part of Nepal and do not represent the whole Nepalese population.

There must be a more detailed study on large sample number from the various parts of the country to generalize the suitability of serum ADA for the early diagnosis of RA in Nepalese population.

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