



# SERUM TOTAL ADENOSINE DEAMINASE ACTIVITY IN NEPALESE PATIENTS WITH RHEUMATOID ARTHRITIS

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## ABSTRACT

### Objective:

Several studies indicate that serum adenosine deaminase (ADA) activity could be a potential marker for the diagnosis of patients with rheumatoid arthritis (RA). However, there has been no such study that could independently verify this finding in Nepali population. The present study therefore aims to measure the total ADA activity in the sera of Nepalese RA patients and verify its diagnostic potential.

### Materials and Methods:

A total of 69 RA patients who visited Universal College of Medical Sciences Teaching Hospital (UCMSTH), Bhairahawa, Nepal for their medical treatment were enrolled for this study. An equal number of age and sex-matched healthy controls were also included in the study. Blood samples were collected from each study subjects and analyzed for serum total ADA, C-reactive protein (CRP) and rheumatoid factor (RF).

### Results:

Serum total ADA activity was found to be significantly ( $p < 0.0001$ ) higher ( $30.0 \pm 10.1$  U/L) in all RA patients compared to healthy controls ( $13.5 \pm 3.6$  U/L). However, no significant difference ( $p > 0.05$ ) in the ADA activity was found between the smokers and non-smoker RA patients. Out of total 69 RA patients, only 16 (23.1%) were positive for CRP and 11 (15.9%) were positive for RF.

### Conclusion:

Measurement of serum total ADA activity could be a reliable marker for the diagnosis of RA in Nepali 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, UCMSTH, Nepal

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*“ADA can be one of the earliest potential biomarker for the diagnosis of RA in conjunction with clinical scenario”*

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## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease and, if not controlled, leads to joint damage, disability, decreased quality of life, and cardiovascular and other co-morbidities. This disease is prevalent in 0.5-1.5% of adult population in the industrialized nations with higher prevalence in women and the elderly<sup>1</sup>. While the exact etiology of RA is not yet fully known, genetic factors, obesity and smoking are believed to be the major risk factors<sup>2,3,4</sup>. It is currently diagnosed on the basis of American Rheumatism Association's (ARA) revised criteria, radiographic changes and routine blood tests for ESR, CRP and RF<sup>5,6</sup>. Early and accurate diagnosis of RA is important for timely clinical intervention to prevent the irreversible joint destruction that otherwise might progress to erosive, destructive, and disabling forms<sup>7,8</sup>. The currently used blood tests are useful to some extent for the diagnosis of RA and provide supporting evidence for radiographical and clinical findings. However, they greatly suffer from poor specificities and sensitivities and therefore cannot be the sole basis for the early diagnosis of RA<sup>9</sup>. Hence, there is still a need for more sensitive and specific diagnostic marker (s) whose detection method is technically reliable, simple and rapid.

One of the potential diagnostic markers that is actively being pursued for RA is adenosine deaminase (ADA, EC 3.5.4.4), an enzyme of the purine metabolic pathway catalyzing the deamination of adenosine to inosine and deoxyadenosine to deoxyinosine in mammalian cells<sup>10</sup>. It is present in serum and mainly in lymphoid tissues, and is required for the maturation and function of T lymphocytes and macrophages<sup>11,12,13</sup>. The enzyme exists in two isoenzyme forms: ADA1 and ADA2, coded by separate genes<sup>14</sup>. The ADA activity considerably increases during inflammatory conditions and is attributed to the increased number of nucleated cells, particularly T lymphocytes and macrophages

<sup>15,16</sup>. RA, being one of the chronic inflammatory diseases, should also be accompanied by increased serum ADA activity. There have been some reports which indeed support this hypothesis and demonstrate the increased serum ADA activity in RA patients<sup>17,18,19,20</sup>. However, there has been no such study among Nepalese population which demonstrates the suitability of ADA as a potential diagnostic marker for RA. The aim of this study was therefore to investigate whether serum ADA activity is also increased in Nepalese patients with RA, and to assess its diagnostic potential for routine diagnosis of RA.

## MATERIALS AND METHODS

**Patients:** This is a hospital based case-control study conducted at the Universal College of Medical Sciences Teaching Hospital (UCMSTH), Bhairahawa, Nepal during July 2009 to March 2010. A total of 69 newly diagnosed patients of RA without any medication and an equal number of age and sex matched healthy controls were enrolled for this study. Informed consent was obtained from each subject prior to enrolment in the study. All the patients enrolled for this study were diagnosed by a rheumatologist based on the ARA revised criteria, radiographic changes, ESR, CRP and RF tests. RA patients with tuberculosis, diabetes mellitus, cardiovascular diseases, HIV/AIDS and patients with other types of musculoskeletal disorders e.g. osteoarthritis, osteoporosis, spinal disorders, severe limb trauma and gouty arthritis were not included in the study. Name, age, sex, hospital number, contact address, family history of RA, and smoking habits of all the study subjects were recorded in a special case proforma. The research and ethical review committee of the UCMS approved this study protocol.

### Sample Collection

Venous blood samples (5 ml) from all study

subjects were 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  $-20^{\circ}\text{C}$  for later analysis or analyzed immediately for total ADA activity, CRP and RF at the clinical biochemistry laboratory of UCMSTH.

#### **Measurement of serum total ADA activity:**

The total activity of serum ADA was assayed with a commercially supplied kit according to the instructions of the manufacturer (Tulip Diagnostic (P) Ltd, Verna Goa, India). The assay was based on the colorimetric method described by Galanti and Guisti (1984)<sup>21</sup>. 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  $37^{\circ}\text{C}$ . The total ADA activity was expressed in international units (U/L).

#### **Detection of CRP**

The presence of elevated CRP level in the serum was detected by a rapid latex agglutination test using a commercially supplied CRP-Latex kit (Span Diagnostic Ltd, Surat, 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 mg/L of CRP in the patient's serum. The test was considered positive when the CRP concentration was above 6 mg/L and negative when it was at 6 mg/L and below.

#### **Detection of RF**

Serum RF was detected by using a commercially supplied RF latex reagent kit (RFCL Limited, Uttarakhand, India). The test was performed as per the manufacturer's instructions and 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 IU/L of RF. The test was considered positive if the agglutination

was observed within two minutes.

#### **Statistical analysis**

All the qualitative and quantitative data were entered into the data sheet and analysed by the Origin 6.1 software. Results were presented as number, percentage and mean  $\pm$  SD. The difference between case and control parameters were compared by student's paired t test (two tailed) and p-values  $\leq 0.05$  were considered significant.

## **RESULTS**

The RA patients enrolled for this study presented with different set of clinical signs and symptoms. About 58 (84%) of the total patients presented with the bilateral involvement of joints pain whereas 11 (26%) patients presented unilateral involvement of joints pain as their major complaints. Forty (58%) patients had chief complaints of morning stiffness which lasted for about 2 hours but rest of other did not show the symptoms of morning stiffness. About 21 (30%) patients had pain in wrist, metacarpal and interphalanges whereas other 48 (70%) patients had pain in knee and ankle joints. The patient group had a mean age of  $48.0 \pm 12.0$  years and included 25 males and 44 females. On the other hand, the control group had 34 males and 35 females with the mean age of  $48.6 \pm 12.1$  years. There were 30 (43.48%) smokers and 39 (56.52%) non-smokers among the RA patients whereas 32 (46.38%) smokers and 37(53.62%) non-smokers among the control groups. Among the 69 RA patients, only 16 (23.1%) were positive for CRP test and 11 (15.9%) for RF test. The mean total ADA activity was significantly ( $p < 0.0001$ ) higher ( $30.0 \pm 10.1$  U/L) in all RA patients as compared to healthy controls ( $14.46 \pm 4.64$  U/L). The differences in ADA activity between the smoker and non-smoker sub-groups within RA patients ( $31.0 \pm 12.0$  vs.  $29.0 \pm 8.2$  U/L) and controls ( $13.2 \pm 3.4$  vs.  $13.7 \pm 3.8$  U/L) were not found statistically significant ( $p > 0.05$ ) (**Table 1**).

## DISCUSSION

RA is likely to affect women approximately two times more than men<sup>22</sup> and 80% of people with RA develop signs and symptoms of the disease at between 35 and 50 years of age<sup>23</sup>. Our study showed that almost 1.8 times more women are affected than males in Nepalese population which is in close agreement to previously accepted value. The mean age of the patients was found to be  $48.0 \pm 11.8$  years, which is similar to previous reports<sup>24</sup>.

Most of the current research on RA appears to be focused on its immunology because of the inflammatory response that causes joint and other injury associated with RA. The T-lymphocyte-driven nature of the disease, involving T cell infiltration into the affected joint leading to recruitment of macrophages and fibroblasts, is well established<sup>25</sup>. However, antinuclear antibodies (ANA) found in 30 to 60% of the patients are not RA specific and are associated with a number of other conditions, such as systemic lupus erythematous and scleroderma<sup>26</sup>. On the other hand, the RF found

in 72 to 85% of adult patients, particularly in high titres, is associated with an advanced, more destructive stage of the disease, thus negating its suitability as a diagnostic marker<sup>27</sup>. Likewise, the CRP has also been shown to have little effect in predicting the incidence of RA<sup>28</sup>. In our study among the 69 RA patients, only 16 were positive for CRP test and 11 for RF test (**Table 1**), illustrating clearly the limited use of these parameters for diagnosis of RA.

A promising candidate for this purpose is ADA, whose increase in total serum activity and that of its isozymes ADA1 and ADA2 have been shown to be correlated with clinical activity of rheumatoid arthritis<sup>18, 19, 20, 29, 30, 31</sup>. In addition, ADA activity in synovial fluids and serum has been reported to enable differential diagnosis of RA from osteoarthritis and reactive arthritis<sup>17</sup>. Against this background, this study was aimed at confirming whether serum ADA levels in Nepalese patients show similar correlation with the disease, in order to evaluate whether ADA can serve as a relatively early biochemical marker to diagnose RA

Table 1: Sexwise distribution, mean age, smoking habits, status of serum CRP, RF and total serum activity of ADA of the study subjects

Study Group	Sex	Mean Age (Years)	Smoking habits (Both sexes)	<sup>¶</sup> CRP +Ve	<sup>‡</sup> RF +Ve	Total ADA Activity (U/L)	Mean Total ADA Activity (U/L)
RA Patients (N=69)	Male (n=25)	48.0 ± 11.8	Smokers (n=30)	9	7	31.0 ± 12.0*	30.0 ± 10.1**
	Female (n=44)		Non-smokers (n=39)	7	4	29.0 ± 8.2	
Controls (N=69)	Male (n=34)	48.6 ± 12.1	Smokers (n=32)	0	0	13.2 ± 3.4*	13.5 ± 3.6
	Female (n=35)		Non-smokers (n=37)	0	0	13.7 ± 3.8	

\*p>0.05; \*\*p<0.0001; <sup>¶</sup>CRP= C-Reactive Protein; <sup>‡</sup>RF=Rheumatoid Factor

in Nepalese patients when taken together with clinical signs and symptoms.

The mean total ADA activity of  $30.0 \pm 10.1$  U/L found in the serum of RA patients in our study is considerably lower than that reported by Sari et al<sup>30</sup> who reported it to be 59 U/L in their sample of patients in India. It is unlikely that the Indian and the Nepalese populations would exhibit such large difference. Such difference was also observed for the healthy controls group, which according to our results showed a mean value of  $13.32 \pm 3.11$  U/L whilst they found it to be  $20.71 \pm 5.63$ . These differences might be due to the variations in the method of estimation, or they may point out a real difference between the populations, confirming the need for studies to further establish the ranges of this and other biochemical parameters that can be used to assess the disease in different populations. Moreover 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 CRP. This study has shown moderate positive correlation between serum ADA activity and disease activity score ( $r=+0.347$ ), however it signifies the association of ADA activity and rheumatoid arthritis. Moreover, our study has also indicated that smoking, which is regarded as one of the etiologic factors of RA, might have role only in triggering the initiation of RA but not in its progression since there is no statistically significant difference in the total serum ADA level between RA patients and control groups.

## CONCLUSION

The clear difference in the values of ADA activity between the RA patients and healthy controls definitely points to its usefulness in diagnosing the disease in the Nepalese population when taken in the context of clinical background data. However,

we must emphasize that this is a hospital based study confined only to south western part of Nepal and do not represent the whole Nepalese population. There must be a more detailed study encompassing the representative population from the various parts of the country to generalize the suitability of serum ADA for the early diagnosis of RA in Nepalese population. We believe that our study results serve as baseline data to plan such studies in future.

## AUTHORS' CONTRIBUTIONS

DRP, NG and AJ designed the study, supervised the laboratory analysis, processed and interpreted the laboratory and patient data, drafted the manuscript. RK, LIS and SM helped in the enrolment of RA patients, collection of blood samples and their diagnosis and treatment. RMS participated in the writing of discussion part and edition of the whole manuscript. All authors have read and approved the final manuscript.

## COMPETING INTERESTS

NG, AJ, RK and LIS are currently employed by the Universal College of Medical Sciences, Bhairahawa, Nepal. RMS is currently pursuing his PhD study at the University of Zurich, Zurich, Switzerland. SM is currently employed Shahid Ganga Lal National Heart Centre, Bansbari, Kathmandu, Nepal. DRP is currently employed by the Manipal College of Medical Sciences, Pokhara, Nepal. All authors declare that they have no conflicts of interest.

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