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SERUM ADENOSINE DEAMINASE, URICACID AND MALONDIALDEHYDE LEVELS IN RHEUMATOID ARTHRITIS

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ABSTRACT

Background: Rheumatoid arthritis (RA) is characterized by inflammation of synovial membrane and oxidative stress has been implicated as mediators of tissue damage. Adenosine deaminase plays an important role in inflammation and uric acid, an endogenous antioxidant has free radical scavenging capacity.

Aims and objectives: 1. To estimate the level of ADA in rheumatoid arthritis patients. 2.To assess the lipid peroxidation product (MDA) & antioxidant status (uric acid) in RA.

Materials and Methods: The study group included a total of 60 subjects of which 30 were RA patients and 30 were healthy controls. Serum ADA, uric acid and MDA levels were analysed. Statistical analysis was performed using independent't'test.

Results: Serum ADA ($p<0.05$) and MDA ($p<0.01$) levels were found to be significantly high in rheumatoid arthritis patients when compared to controls. No significant difference was observed in uric acid level in both groups.

Conclusion: These data suggest that increased serum ADA indicates inflammation and increased MDA indicates oxidative stress in rheumatoid arthritis.

Key words: Rheumatoid arthritis, Adenosine deaminase, Reactive oxygen species, Uric acid, Inflammation.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, multisystemdisease. It affects approximately 1-2 % of world's population. In India alone there are some 10 million people with RA.[1] The etiology of RA is not known, but it is classified as one of the autoimmune diseases. 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.[1]

Adenosine deaminase (ADA, adenosine amino hydrolase E.C. 3.5.4.4) is an enzyme present in all nucleated cells predominantly T cells, involved in the metabolism of purine bases, catalyzing the deamination of adenosine, forming inosine.[2,3,7] 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.[4,17]ADA activity is increased in infectious diseases . (Infectious mononucleosis, leprosy, Tuberculosis, Brucellosis and HIV etc.) It is also elevated in certain autoimmune diseases like SLE , Rheumatoid arthritis, Behcet's disease etc.[6]

Oxygen free radicals / reactive oxygen species have been implicated as mediators of tissue damage in the patients with rheumatoid arthritis.[9,10] Recent studies provide evidences for the elevation of lipid peroxides which include malondialdehyde, in rheumatoid arthritis.[8,9]

Uric acid is an aqueous, endogenous antioxidant with 10 fold higher concentration in plasma, than vitamin C and vitamin E. It contribute **2/3** rd of free radical scavenging capacity in plasma.[4] A recent study indicated that increased oxidative stress and/or defective antioxidant status contribute to the pathology of rheumatoid arthritis.[9,12]

The present study is an attempt to estimate the levels of serum adenosine deaminase, malondialehyde and uric acid that represent the oxidant - antioxidant status, in rheumatoid arthritis patients.

MATERIALS AND METHODS

The present case control study was undertaken in the department of Biochemistry, Vinayaka Mission's Kirupananda VariyarMedical College, Salem, India. The study comprised of 30 rheumatoid arthritis cases and 30 age and sex matched controls. RA patients diagnosed according to the revised criteria formulated by

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the American college of Rheumatology, who were attending out patient department of Rheumatology, Vinayaka city centre hospital, Salem were selected as cases. The patients with age around 20- 60 years were selected for this study.

Subjects with osteoarthritis, inflammatory diseases, SLE, coronary artery disease, diabetes mellitus, infectious diseases and smokers were excluded from the study.The study has been approved by the ethical committee of VMKVMC. Informed consent was obtained from all the subjects. 5 ml of venous blood was collected for estimation of ADA, C-reactive protein(CRP), Rheumatoid factor(RF), Malondialdehyde (MDA), Erythrocyte sedimentation rate (ESR) and Uric acid.

The serum was immediately assayed for ADA activity by spectrophotometric method (Tulip diagnostics-ADA-MTB kit). CRP was determined by TUBI.MAGIWEL ELISA kit method. ESR was determined byWestergren method in whole blood with EDTA. RF and MDA were measured respectively by using Turbilatex method and TBARS assay kit method.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 16. Independent sample 't' test was done to compare the means between the 2 groups. Correlation was done by using Pearson's correlation. P < 0 .01 is considered as statistically significant.

RESULTS

A total of 60 age and sex matched subjects were recruited for the study. Among them, 30 were rheumatoid arthritis patients (Group1) and 30 belonged to the control population(Group2).

All the RA patients included in the present study had positive RF. ESR was elevated (**46.60 ± 3.12 mm/hr**) and serum CRP levels were significantly raised (9.10 ± 1.15mg/dl) in RA patients (p<0.01) when compared to that of normal healthy control individuals. Serum ADA levels were significantly elevated in rheumatoid arthritis patients (20.54 ± 3.21 U/L) and no significant change in uric acid in rheumatoid arthritis. (Table 1)

In table 2, A significant positive correlation was observed between ADA and MDA, CRP(p<0.01), ESR(p<0.05) levels in RA patients. There is no significant correlation between ADA and uric acid.

Table:1. Mean and standard error for the 5 parameters among RA patients and controls

PARAMETERS	Group 1 (Rheumatoid arthritis)	Group 2 (controls)
ADA (U/L)	20.54 ± 3.21**	9.80 ± 0.53
URIC ACID(mg/dl)	4.08 ± 0.24^{NS}	4.28 ± 0.28
MDA (nmol/ml)	13.828 ± 1.54**	4.67 ± 0.37
CRP (mg/dl)	9.10 ± 1.15**	1.55 ± 0.72
ESR (mm/hr)	46.60 ± 3.12**	12.8 ± 0.95

(NS- Non significant ; **P<0.01)

Table:2. Correlations between ADA and other parameters in patient group

PARAMETERS	ADA
URIC ACID (mg/dl)	− .224
MDA (nmol/ml)	.752**
CRP (mg/dl)	.575**
ESR (mm/hr)	.355*

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

In the present study, variations in the levels of adenosine deaminase, malondialdehyde and uric acid in rheumatoid arthritis patients from that of the healthy controls have been evaluated.

In our study, serum ADA levels were significantly increased in RA patients compared to healthy individuals who formed the control group.This is in accordance with Sari et al. , who have considered ADA as an additional marker in the RA diagnosis.[2,17]

Significantly raised levels of ESR and CRP in these patients is suggestive of inflammatory response in rheumatoid arthritis. A significant positive correlation was observed between ESR, CRP and ADA suggesting significant role of adenosine deaminase in inflammation in rheumatoid arthritis patients.

RA is characterized by persistent inflammation in the synovial membranes of joints, associated with migration of activated phagocytes**, T lymphocytes** and other leukocytes into synovial and periarticular tissue.[1,2,3] Accumalated T lymphocytes liberate adenosine deaminase into synovial fluid and extra cellular fluid. Increased ADA causes activation of neutrophils, which

results in increased generation of reactive oxygen species (ROS) like O_2^- and H_2O_2.[6] These ROS attack the membrane lipids (PUFA) causing lipid peroxidation, which may play an important role in cartilage damage and tissue injury in rheumatoid arthritis patients and accentuating further joint inflammation in RA.[9,13,15]

We have found a significant increase in serum MDA levels in rheumatoid arthritis patients compared to the controls and a positive significant correlation between ADA and MDA levels in rheumatoid arthritis patients, indicating the major role played by lipid peroxidation in joint inflammation in this disease. This was accordance with study conducted by VasanthiPallanti.

We have also estimated the levels of uric acid which is a protective antioxidant, particularly effective in quenching hydroxyl, superoxide and peroxinitrite radical , thereby preventing lipid peroxidation.[4,5,16] We have not found any significant change in the uric acid level in the rheumatoid arthritis patients compared to the healthy individuals. This is contradicted by Ajay Kumar Singh et al.who reported icreased uric acid level in RA patients. Few studies support our findings.[4,9]

Though we expect an increase in uric acid levels due to increase in adenosine deaminase levels in rheumatoid arthritis patients , our study showed normal uric acid levels. This is probably may be due to increased utilization of uric acid in trapping the free radicals produced and its conversion to allantoin.[4]

CONCLUSION

Serum adenosine deaminase levels has been found to be increased in rheumatoid arthritis patients, which indicates activation of cell mediated immunity. Increase in the level of malondialdehyde indicates the important role played by ROS , in tissue damage and joint inflammation in this disease. Perhaps reduction in lipid peroxidation and supplementation with antioxidant therapy may open new avenues in the treatment of rheumatoid arthritis.

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