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Oxidative Stress Status, Inflammatory Markers and their Association with Disease Activity Score in Rheumatoid Arthritis Patients of Tamilnadu Population

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Abstract

Background: Morbidity and mortality rates are higher in individuals with rheumatoid arthritis than in the general population. Cardiovascular diseases now represent one of the most common causes of death in Rheumatoid arthritis. It appears to represent an independent risk factor for ischemic heart disease. Rheumatoid arthritis 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. The aim of this study is to estimate the serum levels of ADA, MDA, uric acid and CRP levels to know the oxidative stress status in rheumatoid arthritis patients. *Methods:* This is a prospective case control study consists a total of 100 subjects of which 50 were RA patients and 50 were healthy controls. Serum ADA, MDA, uric acid levels and ESR were analyzed in both controls and cases. Statistical analysis was performed using independent 't' test. *Results:* Serum uric acid, ADA, MDA, CRP, ESR ($p<0.0001$) levels were found to be significantly high in rheumatoid arthritis patients when compared to controls. There is a strong positive correlation of biochemical parameters with disease activity score 28 (DAS28) in rheumatoid arthritis patients. *Conclusion:* In the present study increased serum uric acid, ADA, MDA, CRP levels and ESR clearly suggest their role in causing inflammation and increased oxidative stress in rheumatoid arthritis patients.

Keywords: Rheumatoid Arthritits; C - Reactive Protein; Uric Acid; Adenosine Deaminase; Oxidative Stress; Malondialdehyde; Erythrocyte Sedimentation Rate (ESR).

Introduction

Rheumatoid arthritis (RA) is the most common auto immune inflammatory diseases. It is characterized by chronic inflammation, which affects multi organs. It affects approximately 1-2 % of world's population [1]. In India alone approximately there are 10 million people suffering from RA. There is a prominent immunological dysfunction in the joints and many other tissues by accumulation of T and B lymphocytes, monocytes and macrophage [1,2]. Rheumatoid factor consists of antibodies directed against the Fc region of human IgG [3,4]. They are the hallmark of Rheumatoid Arthritis and can be detected in 60-80% of Rheumatoid Arthritis patients [4,5]. C-reactive protein (CRP) is a member of the class of acute phase reactants, which is synthesized exclusively in the liver. Its levels have been shown to be raised by many folds following acute inflammation [1,3, 5].

Accordingly, CRP has been suggested to be a marker of inflammation in several conditions including psoriasis, rheumatoid arthritis, tuberculosis, cancer, and myocardial infarction [1,2].

Adenosine deaminase (ADA) 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 [6]. 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. ADA activity is increased in infectious diseases like leprosy, Tuberculosis, Brucellosis and HIV etc [7]. It is also elevated in certain autoimmune diseases like Rheumatoid arthritis, Systemic lupus erythematosis (SLE), Behcet's disease etc [6,7]. Uric acid is an end product of the metabolism of purine nucleotides that are the principal constituents of cellular energy store such as ATP and components of DNA and RNA. It contribute 2/3 rd of free radical scavenging capacity in plasma [8]. Recent studies have shown that hyperuricaemia is associated with hypertension, systemic inflammation and cardiovascular disease mediated by endothelial dysfunction and pathologic vascular remodeling [9]. A recent study indicated that increased oxidative stress and/or defective antioxidant status contribute to the pathology of rheumatoid arthritis. Oxygen free radicals/reactive oxygen species have been implicated as mediators of tissue damage in the patients with rheumatoid arthritis [8,9,10]. Recent studies provide evidences for the elevation of lipid peroxides which include malondialdehyde (MDA), in rheumatoid arthritis [11]. The primary aims of the study are to assess lipid peroxidation, oxidative stress status, high sensitivity C-reactive protein level, serum uric acid level at diagnosis and association of these parameters with disease activity score.

Materials and Methods

The study includes 50 newly diagnosed rheumatoid arthritis cases along with 50 age and BMI matched controls. The patients with age around 32 - 56 years were selected for this study. Patients with known history of diabetes, hypertension, liver, kidney, thyroid dysfunction, any previous or current cardiac illness or hyperuricemia, or with coexisting disease associated with high uric acid levels e.g., malignancy, chronic renal failure, active chronic infections e.g. Tuberculosis were excluded. Diagnosis of Rheumatoid Arthritis was done according to the revised criteria formulated by the American college of Rheumatology [12]. Besides biochemical investigations such as serum uric acid, ADA, MDA, CRP, erythrocyte sedimentation rate (ESR), disease activity score (DAS) based on 28 joint counts such as swollen joint count +tender joint count +patient's global activity was also considered, at diagnosis[12]. For all the patients RA factor qualitative test is positive. The study was approved by Institutional Ethics Committee. Written and informed consent was obtained from all the participants.

All the study subjects (both cases and controls) underwent biochemical analysis of Serum RA factor, Serum C-reactive protein (CRP), Serum Uric acid, adenosine deaminase (ADA), malondialdehyde (MDA) and erythrocyte sedimentation rate (ESR). About 5 ml of venous blood was collected serum separated by centrifuging at 3500 rpm for 15 minutes. Serum was immediately assayed for ADA activity by semi automated biochemistry analyser using, Tulip diagnostics-ADAMTB kit commercially available in the market. RA factor was done by qualitative slide test by using Tulip diagnostics kit.

Serum Uric acid was estimated by Uricase/ Peroxidase method using Coral, Tulip diagnostic kit, CRP was determined by turbidimetric immunoassay using Erba XL Sys pack kit and MDA was measured respectively by using TBARS assay kit method. ESR was determined by Westergren method in whole blood with EDTA.

Results obtained were tabulated and expressed as mean±standard deviation for continuously distributed variable, and in absolute numbers. Pearson correlation was done to find out the association of biochemical parameters with disease activity score (DAS28). A p value <0.05 was considered statistically significant. SPSS version 16.0 software was used for statistical analysis.

Results

A total of 100 age and BMI matched subjects were recruited for the study among them, 50 were newly diagnosed rheumatoid arthritis patients and another 50 apparently healthy individual belonged to the control population.

Mean age of the controls is 43.2±6.6 years and that of RA cases is 42.7±6.7 years. All the RA patients included in the present study had positive RA factor. ESR was elevated (58.4±14.7 mm/hr) and serum CRP levels were significantly raised (7.4±2.1 mg/dl) in RA patients ($p<0.0001$) when compared to that of normal healthy control individuals (Table 1). Serum ADA levels were significantly elevated in rheumatoid arthritis patients (32.7±7.1 U/L) and also significant change in uric acid in rheumatoid arthritis (Table 1).

It is evident from Table 2, that there is a significant positive correlation between ADA and MDA, ADA vs CRP ($p<0.01$), ADA vs ESR ($p<0.05$) levels in RA patients. It is also observed that there is a significant positive correlation of uric acid vs ADA, uric acid vs CRP and uric acid vs ESR. These observations were tabulated in Table 1 and 2 and depicted graphically in Figure 1. The determined Pearson correlation coefficient between the values ADA, MDA, CRP, ESR are demonstrated in Table 2. There is a strong positive correlation between uric acid and ADA ($r = 0.78$; $p <0.01$). A positive correlation of uric acid with CRP and ESR is observed in this study ($r = 0.32$, $p <0.05$; $r = 0.35$, <0.05 respectively). There is strong positive correlation observed between CRP and ESR ($r = 0.80$, $p <0.01$). Similarly there is positive correlation observed with Disease activity score was calculated based on 28 joint examinations and symptoms like swollen joint count, tender joint count, patient's global activity [12]. In the present study DAS 28 score is 3.9 ± 1.0. DAS 28 and ADA, CRP, ESR ($r = 0.41$, $p <0.05$; $r = 0.59$, $p <0.05$; $r = 0.67$, $p <0.01$).

Table 1: Comparison of all biochemical parameters in controls and cases

Parameter	Controls (n = 50)	Cases (n = 50)	P value
Age (Years)	43.2 ± 6.6	42.7 ± 6.7	0.70 NS
Height (cm)	161.4 ± 7.2	160.7 ± 6.7	0.61 NS
Weight (Kg)	54.2 ± 6.5	54.5 ± 7.9	0.83 NS
BMI (Kg/m^2)	20.7 ± 1.7	21.0 ± 2.8	0.51 NS
ADA (U/L)	17.4 ± 3.7	32.7 ± 7.1	0.0001*
MDA (nmol/ml)	4.7 ± 0.6	7.3 ± 3.0	0.0001*
Uric acid (mg/dl)	5.7 ± 0.9	7.2 ± 1.3	0.0001*
CRP (mg/dl)	2.9 ± 1.2	7.4 ± 2.1	0.0001*
ESR (mm/hour)	18.1 ± 5.8	58.4 ± 14.7	0.0001*

NS – not significant; *p value is significant.

Table 2: Pearson correlation for studied parameters in Rheumatoid Arthritis cases

	BMI	Uric acid	ADA	MDA	CRP	ESR
BMI	1	-	-	-	-	-
URIC ACID	0.09	1	-	-	-	-
ADA	0.19	0.78**	1	-	-	-
MDA	-0.02	-0.01	-0.26	1	-	-
CRP	-0.01	-0.32*	0.05	0.25	1	0.80**
ESR	-0.2	0.35*	0.14	-0.01	0.80**	1
DAS 28	0.23	0.34*	0.41*	0.52*	0.59*	0.67**

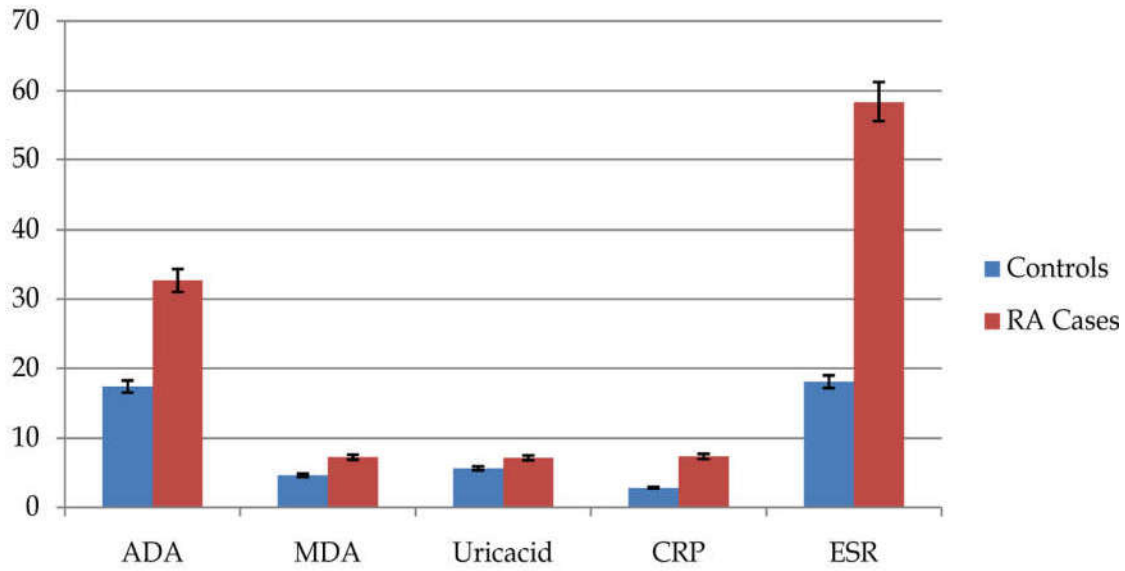

Fig. 1: Comparison of Biochemical parameters ADA, MDA, Uric acid, CRP and ESR among controls and Rheumatoid Arthritis cases

Discussion

In the present study, variations in the levels of serum adenosine deaminase, malondialdehyde, CRP, ESR, and uric acid in rheumatoid arthritis (RA) patients and that of the apparently healthy individuals taken as controls have been studied. This is a prospective study conducted to know the association of uric acid and oxidative stress in rheumatoid arthritis (RA).

CRP is marker for systemic inflammation. In this study, it is observed that systemic inflammation (Serum CRP) is an independent and strong determinant of endothelial dysfunction & microvascular circulation in patients with Rheumatoid Arthritis.

The source of circulating CRP has been thought to be the hepatocyte [13]. However, it has been recently reported that human vascular cells produce CRP in response to inflammatory cytokines [14]. It has also been observed that uric acid induces changes in vascular proliferation and function mediated by de novo production of CRP In human vascular cells [14, 15]. Serum CRP level was most closely correlated with the activity of the disease in Rheumatoid Arthritis patients. Previous investigators have also reported positive correlation between CRP levels and disease activity in RA Patients [13-15].

RA is characterized by systemic inflammation especially in the synovial membranes of joints, associated with migration of activate phagocytes and lymphocytes into synovial and periarticular tissue. Accumulated T lymphocytes liberate adenosine deaminase into synovial fluid and extra cellular fluid. Increased ADA causes activation of neutrophils [2,7-9,12,13], which results in increased generation of reactive oxygen species (ROS) like O_2 and H_2O_2.

These ROS attack the membrane lipids 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 [2,8-10,12,14].

Although, different studies have reported different normal values for serum ADA level at 37°C, for this study the normal range reference for ADA activity was considered as 14-22 mmol/l [16,17]. The mean value for ADA activity in our patients was 32.7±7.1 which is significantly higher than the normal ones compared with other studies (p value <0.05). In our study, serum ADA levels were significantly increased in RA patients compared to control group (Table 1). This is in accordance with the previous studies who have considered ADA as an additional marker in the RA diagnosis [5-7,18]. Significantly elevated levels of ESR and CRP in RA patients, is suggestive of inflammatory response in rheumatoid arthritis.

It is clear from the results of this study that increase in serum MDA levels in rheumatoid arthritis patients is due to oxidative stress which is responsible for lipid per-oxidation.

Serum MDA levels are increased in RA patients compared to the controls (0.0001) (Table 1). A significant increase in serum MDA levels in rheumatoid arthritis patients, indicating the major role played by lipid peroxidation in joint inflammation in this disease. These results are in accordance with previous results reported by Erkilic K et al [6], Nakamachi Y et al [7], Pallanti V et al [11], Skogh T et al [15], Salesi M et al [16].

In the present study increased oxidative stress, is reflected by increased serum levels of MDA (measure of lipid peroxidation) in peripheral blood of patients with RA. These results are also in accordance with the earlier studies [19]. MDA, the product of lipid peroxidation reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. The longer chain polyunsaturated fatty acids are especially potent at increasing lipid peroxidation and causing cell damage by oxidative stress [20].

A significant positive correlation was observed between biochemical parameters (Uric acid, ADA, MDA, CRP, ESR,) with DAS 28 (DAS 28 vs Uric acid r=0.34*, p <0.05; DAS 28 vs ADA r = 0.41*, p <0.05; DAS 28 vs MDA r = 0.52*, p <0.05; DAS 28 vs CRP r = 0.59*, p <0.05; DAS 28 vs ESR r = 0.67**, p<0.01) suggesting significant role of uric acid, adenosine deaminase, MDA, CRP in causing inflammation in rheumatoid arthritis patients (Table 2). These results are on par with the previous studies reported by Pallanti V et al [11], Ghosh A et al [12], Skogh T et al [19], Scot DL et al [20].

Serum uric acid which is a pro-oxidant, particularly effective in quenching hydroxyl, superoxide and peroxynitryl radical, thereby preventing lipid peroxidation [8,10]. In the present study it is found that there is a significant increase in the uric acid level in the rheumatoid arthritis patients when compared to the healthy controls. This is in accordance with the previous studies reported by Panoulas VF et al [21], Lippi G et al [22]. There are few studies which contradict with our findings [5, 17, 23].

Conclusion

From the present study it can be concluded that increased serum ADA causes the activation of cell mediated immunity which in turn results in tissue damage and joint inflammation by increase in MDA indicates oxidative stress in rheumatoid arthritis. Biochemical alteration like increased uric acid, CRP, ADA, ESR levels in patients reflects the pathogenesis of RA. It is also evident from the results of this study that lipid peroxidation is a giant distracter in rheumatoid arthritis. Increased oxidative threat in rheumatoid arthritis is evidenced by raised serum MDA levels. May be reduction in lipid peroxidation and supplementation of antioxidants may open new ways in the treatment of rheumatoid arthritis.

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