Evaluation of Antioxidants and Lipid Peroxidation Status in Rheumatoid Arthritis Patients

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Abstract:Background and Objectives:Rheumatoid Arthritis (RA) is characterized by a chronic hypertrophic synovitis leading to destruction of connective tissues and functional damage of cartilage and bony structure. Reactive oxygen species play an important role in tissue injury of this disease. The objective of this study was to measure the oxidative stress, enzymatic and non-enzymatic antioxidants in both study and control groups. Materials and methods: The study was conducted on 50 RA patients (35 Female /15 Male) with mean age of (46±6.3) years. Both the study groups were non-smokers and non-alcoholics and were not suffering from any other chronic disease. Lipid peroxidation products, enzymatic and non-enzymatic antioxidants were estimated in cases (50) and controls (30). Results: TBARs levels in patients with Rheumatoid arthritis were found to be significantly ($p<0.001$) higher than controls, whereas levels of GSH-Px, SOD and CAT, Vitamin E ($p<0.001$) and vitamin C ($p<0.004$) were significantly lower in the RA patients compared to control subjects. Uric acid and ceruloplasmin levels in the plasma of RA patients were significantly ($p <0.001$) higher as compared to control subjects. Conclusion: The enhanced lipid peroxidation accompanied by perturbation in antioxidant status indicates free radical mediated oxidative damage in rheumatoid arthritis.[Alam R et al NJIRM 2013; 4(5) : 1-5]

Key Words: Antioxidants, Free radicals, Lipid peroxidation,Rheumatoid Arthritis

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Introduction: Rheumatoid Arthritis (RA) is a chronic multisystem autoimmune disease of unknown etiology which affects approximately 1-2% of the total world population.[1] It is characterized by chronic inflammation of the joints and tissues around the joints with infiltration of macrophages and activated T-cells.[2-4]

Free radicals, the highly reactive entity and short lived molecules, are constantly produced in a wide variety of normal physiological functions. They exert beneficial effects on cellular responses and immune functions at low and moderate levels. At high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures.[5-14]Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders and neurodegenerative diseases. Oxidative damage and inflammation in various rheumatic diseases were proved by increased levels of isoprostanes and prostaglandins in serum and synovial fluid compared to controls.[15]

The harmful effect of Reactive oxygen species is neutralized by a broad class of protective agents termed antioxidants which prevents oxidative damage by reacting with free radicals before any other molecules can become a target. The non-enzymatic antioxidants (Vitamin C, E and glutathione) and enzymatic antioxidants (SOD, CAT and GSH-Px) play an important role in the protection of cells and tissues against free radical mediated tissue damage.[16,17] It has been postulated that dietary intervention with vitamins may reduce oxidative stress and thereby reduce the disease related morbidity and mortality.[18]

The present study was designed to assess the association between oxidative stress and the concentration of various enzymatic and non-enzymatic antioxidants in rheumatoid arthritis patients as well as inflammatory and biochemical parameters in patients with rheumatoid arthritis and healthy age and sex matched controls.

Materials& Methods: The present study was conducted on 50 RA patients (female- 35, male-15) with mean age of (46±6.3) years and the control group consisted of 30 healthy individuals with mean age of (42±4.8) years. All the cases met the revised criteria for rheumatoid arthritis of the American College of Rheumatology. The study was approved by the Institute Ethics Committee, Index Medical College Hospital and Research Centre,

Indore, India and informed consent was obtained from all the cases and control subjects. Both the study groups (patients and control) were non-smokers and non-alcoholics and were not suffering from any other chronic disease.

Blood samples were collected in vacutainers with anticoagulant EDTA and in plain vacutainers without any anticoagulant from RA patients and control subjects. The plasma from EDTA anticoagulated blood was separated, immediately stored at -80ºC and used for determination of MDA, ceruloplasmin, Vitamin C, Vitamin E, GSH-Px, catalase, SOD and uric acid. Lipid peroxides were estimated by TBARs in plasma by the method of Yagi.[19] Ceruloplasmin was measured by the method of Ravin.[20] The levels of non-enzymatic antioxidant Vitamin C was estimated by the method of Roe and Kuther.[21]Vitamin E by the method of Desai[22]and Uric acid was estimated by standard kit method (ERBA diagnostics Mannheim GmbH). Enzymatic antioxidants were measured by the method of Rotruck et.al.[23] for GSH-Px, SOD by the method of Kakkar[24] and catalase by the method of Sinha.[25]ESR was measured by Wintrobe tube method.[26]Hemoglobinand hematocrit values wereestimated by Sysmex XS 800i clinical hematology autoanalyzer, Transasia, Mannheim GmbH.RA factor was detected by using Rheumatoid Factor Latex Test Kit in the serum of both study groupsby Beacon, India. All reagents and chemicals used were of analytical grade and were purchased from Merck Chemical Co., Germany.

All data were expressed as mean ±SD. Unpaired student's t-test was used for between group comparisons. Differences were considered of statistical significance when the p-value was $p<0.05$.

Results: The study was conducted on 50 Rheumatoid Arthritis patients (both male/female) mean age 46±6.3years. The demographic and clinical parameters in Rheumatoid Arthritis patients and control subjects are listed in Table 1. The decreased BMI in Rheumatoid Arthritis patients (21.2±2.10 kg/m2) when compared to control (23.2±2.50 kg/m2). RA factor is positive with titers value (117±24.56) in Rheumatoid Arthritis patients while control groups were seronegative. ESR was significantly higher in Rheumatoid Arthritis patients ($p <0.001$) while hemoglobin and hematocrit values were significantly lower in Rheumatoid Arthritis patients ($p <0.001$) as compared to control subjects.

Table 1: Demographic and Clinical Parameters in Rheumatoid Arthritis Patients & Control Subjects

Parameters	Rheumatoid Arthritis Patients	Controls	P-value
	n=50	n= 30	
Age (years)	46±6.3	42±4.8	-
Sex (M/F)	15/35	07/23	-
BMI (kg/m^2)	21.2±2.10	23.2±2.50	-
Duration of disease (years)	8±2.20	-	-
RA factor (IU/ml)	117±24.56 (Positive)	Negative	-
ESR (mm/hour)	36±10.5	12±2.65	<0.001
Hemoglobin (gm/dl)	10.61±1.4	12.52±1.5	<0.001
Hematocrit (%)	34.5±3.5	41.2±4.2	<0.001

Values are expressed as mean± S.D n= number of subjects; 'P' <0.05 was considered significant.

Table 2 shows the enzymatic, non-enzymatic and lipid peroxidation status in the plasma of Rheumatoid arthritis patients and control subjects. The levels of TBARs in Rheumatoid arthritis patients were significantly increased ($p <0.001$) as compared to healthy subjects. The levels of GSH-Px, SOD, CAT, Vitamin E and Vitamin C ($p <0.004$) in the plasma of Rheumatoid arthritis patients were significantly decreased ($p <0.001$). Uric acid and ceruloplasmin levels in the plasma of Rheumatoid arthritis patients were significantly increased ($p <0.001$) as compared to control subjects.

Table 2: Enzymatic, non-enzymatic antioxidants & lipid peroxidation status in the plasma of Rheumatoid arthritis patients and controls.

Parameters	Rheumatoid arthritis patients	Controls	P-value
	n= 50	n= 30	
TBARs (nmol/ml)	7.09±0.52	3.87±0.41	<0.001
Glutathione peroxidase (U^a/ml)	0.235±0.018	0.265±0.025	<0.001
Catalase (U^b/ml)	1.56±0.13	1.89±0.20	<0.001
Superoxide dismutase (U^c/ml)	4.93±0.64	5.98±0.78	<0.001
Vitamin C (mg/L)	11.30±1.71	12.55±1.98	<0.004
Vitamin E (mg/dl)	0.92±0.12	1.48±0.20	<0.001
Uric acid (mg/dl)	6.21±1.30	4.25±0.78	<0.001
Ceruloplasmin (mg/dl)	35.41± 2.85	25.85±2.40	<0.001

Values are expressed as mean± S.D, n= number of subjects; 'P'<0.05 was considered significant.

[a] moles of reduced glutathione utilized/minute.

[b]moles of hydrogen peroxide consumed/minute.

[c]50% inhibition of NBT reduction.

Discussion: Rheumatoid arthritis (RA) is a chronic systemic disease, usually characterized by inflammation of multiple joints. In this study levels of ESR, RA factor, lipid peroxide, enzymatic and non-enzymatic antioxidant status was assessed in both study (RA patients) and control groups. Excessive oxidative stress plays an important role in the pathogenesis of autoimmune diseases by enhancing the inflammation, inducing apoptotic cell death, and breaking down the immunological tolerance.[27] Lipid peroxides that are generated at the site of inflammation of tissue injury diffuse into blood and can be estimated in serum or plasma, which reflect the severity of tissue damage.[28] The lipid peroxide (LPO) products, especially MDA, have been established as indicators of lipid peroxidation.[29]

The present study shows significantly high levels of MDA in RA patients as compared to control. Our findings are in accordance with Shaabani et.al.[30] and Ansari and Jaiswal.[31] Elevated levels of lipid peroxidation or MDA may be due to an imbalance between defence mechanisms and free radical generation process. In our study GSH-Px, SOD and catalase activity in plasma were lower in the patients than in control group. These findings are in accordance with Hassan et.al.[32], Bae et.al [33] and Sarban et.al.[34] respectively. The disease itself may inhibit the activity of the SOD. The decreased levels of antioxidants in RA patients is due to the inactivation of the enzymes by H_2O_2 and suggests that these enzymes play an important role in the rheumatic process and increased oxidative stress. Ceruloplasmin, Cu containing protein, has been found to be increased in RA patients as compared to control. Increased levels of Ceruloplasmin may be related to its scavenging action of superoxide radicals that are generated during the inflammatory process of RA.

Significantly lower levels of Vitamin E and Vitamin C were found in RA patients as compared to control group. Vitamin E helps to trap free radicals and interrupt the chain reaction that damage the cells. Regeneration of Vitamin E depends on Vitamin C. As there is an increased oxidative stress in RA there may be increased consumption of Vitamin C and Vitamin E. This reduction in ascorbate levels suggests its role in combating oxidative stress. Uric acid levels were higher in RA patients than in control this is in accordance with the results of Mahajan et.al.[35]

Conclusions: Overproduction of free radicals by the inflammatory processes of rheumatoid arthritis causes concomitant failure of antioxidant defence mechanism. Thus there occurs an imbalance between ROS production and the antioxidant defence system in inflammatory RA disease.

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