

Oxidative stress and enzymatic antioxidant status in rheumatoid arthritis: a case control study

PRAKASH B. DESAI, S. MANJUNATH, SUMANGALA KADI,
K. CHETANA, J. VANISHREE

Department of Biochemistry, J.N. Medical College, Belgaum, Karnataka (India)

Abstract. – Background and Objectives: Rheumatoid arthritis (RA) is a chronic multisystem disease of unknown cause. Formation of reactive oxygen species and lipid peroxides as a result of disease activity may play an important role. Oxidative stress and decreased antioxidant status are the hallmarks in patients of RA as observed in recent years. The objectives of the study was to determine oxidative stress by measuring malondialdehyde and enzymatic antioxidant status by estimating superoxide dismutase and glutathione reductase in patients of RA and then comparing with healthy individuals.

Materials and Methods: The present study comprises of 40 RA cases who were clinically diagnosed and confirmed by laboratory tests, attending KLE Society's Dr Prabhakar Kore Hospital and Research Centre, Belgaum. Southern India attached to J.N. Medical College. between September 2007 to August 2008. All the patients were in the age group of 40-60 years including both the sexes. Biochemical parameters like malondialdehyde, enzymatic antioxidants like superoxide dismutase and glutathione reductase were estimated in cases (40) and controls (40).

Results: The mean level of blood malondialdehyde (in nmol/ml) in controls was 6.19 ± 0.96 and 11.48 ± 0.76 in cases. The mean level of superoxide dismutase (IU/g of Hb) in controls was 948.32 ± 99.88 and 443.68 ± 111.69 in the cases. The mean glutathione reductase level (in IU/g of Hb) in controls was 8.91 ± 1.04 and 2.96 ± 0.79 in the cases.

Conclusions: This study revealed that there was an increased oxidative stress and a decreased antioxidant defense in patients with rheumatoid arthritis.

Key Words:

Rheumatoid Arthritis (RA), Oxidative stress, Malondialdehyde, Superoxide dismutase, Glutathione reductase.

Introduction

Rheumatoid arthritis (RA) is a chronic multisystem disease of unknown cause which affects

approximately 1-2% of the total world population. Annual incidence of 0.5% to 1% of total world population is reported every year, both in developed and developing countries. Women are affected more than men. The onset is more frequent during the fourth and fifth decades of life, with 80% of all patients developing the disease between the ages 35 and 50 years.

The characteristic feature of RA is non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues and joint deformities. Although there are a variety of systemic manifestations, the characteristic feature of established RA is a persistent inflammatory synovitis usually involving peripheral joints in a symmetric distribution¹. Rheumatoid Arthritis is not only a polyarthritis, and the name *rheumatoid disease* is preferable since it directs attention to the whole patients and not just the joints².

Genetic risk factors do not fully account for the incidence of RA. Undoubtedly multiple factors responsible for amplifying and perpetuating the initial pathology are now recognized. It may well be known that there is no single etiologic factor and that many similar or diverse stimuli brought to bear upon the susceptible host may trigger reactions leading to the clinical syndrome which we now recognize as RA.

An exogenous infection or molecular components of an infectious agent are likely candidates for the primary etiologic agent. The puzzle of whether antibodies to host components have a casual relationship to disease or whether they are simply another manifestation of it remains unsolved.

The earliest abnormality in RA is an increase in blood flow to the synovium. Increased permeability of vessels leads to increased accumulation of synovial fluid and superficial synovial lining cells proliferate. However, the proliferative synovitis can be silent and destroy joints without outward signs of inflammation.

Exact reason behind bone erosion and joint deformities is not fully understood. Many investigators have focused on oxidative stress since last few years and suggest that RA patients are more prone to lipid peroxidation. Lipid peroxidation occurs as a result of free radicals generated in the body. Free radicals and free radical derived oxidants play important roles in biological system and have been implicated in the pathology of many diseases.

A free radical, in contrast, is a molecule or molecular fragment that contains one or more unpaired electrons in the outer orbital³. Free radicals are produced in the body due to leak in the electron transport chain, inflammatory conditions, ionizing radiations, drugs, chemical toxins, etc.

Oxidation reactions ensure that molecular oxygen is completely reduced to water. The products of partial reduction of oxygen are highly reactive and make havoc in the living systems. Hence they are also called reactive oxygen species (ROS) ex: superoxide anion radical (O_2^-), hydroperoxy radical ($HOO\cdot$), hydroxyl radical ($OH\cdot$), etc.

Extreme reactivity, short life span, generation of new ROS by chain reaction and damage to various tissues are the important characteristics of the ROS.

Lipid peroxidation and consequent degradation products, such as malondialdehyde (MDA), are a result of free radicals generated in the body.

The damage produced by ROS may be prevented by antioxidants. There are two types of antioxidants.

Preventive antioxidants: which will inhibit the initial production of free radicals, e.g. catalase.

Chain breaking antioxidants: which once the peroxy radicals are generated they can inhibit the propagative phase ex superoxide dismutase (SOD), vitamin E, etc.

It is possible that, generation of ROS may be particularly important factor for bone resorption in inflammatory process. Human selectins mediate the inflammatory responses in rheumatoid arthritis, asthma, psoriasis, multiple sclerosis and the rejection of transplanted organs and thus there is great interest in developing drugs that inhibit selectin mediated cell adhesion⁴.

In recent years it has been shown that oxidative stress and antioxidants play an important role in the disease process of RA. So, the present study was undertaken to assess oxidative stress by measuring malondialdehyde and antioxidant

status by estimating superoxide dismutase and glutathione reductase levels in rheumatoid arthritis patients.

Materials and Methods

Source of Data

The present study comprises of 40 RA patients (males-20 and females-20) from KLE Society's, Dr. Prabhakar Kore Hospital and Medical Research Centre, attached to J.N. Medical College, Belgaum, India. The study was undertaken between September 2007 to August 2008. All the patients were in the age group of 40-60 years and were not on any nutritional supplements. Age and sex matched 40 healthy individuals served as controls.

Criteria for the Selection of the Cases

Inclusion criteria: Clinically diagnosed cases of rheumatoid arthritis and confirmed by laboratory tests.

Exclusion criteria: Osteoarthritis, tubercular arthritis, infective arthritis, rheumatic fever, pulmonary tuberculosis, pneumonia, costochondritis (Tietze's disease), arthritis other than RA fitting into any syndrome, chronic smokers, chronic alcoholics and any other systemic disease.

Informed consent was taken from all the cases and the study was approved by the Ethical and Research Committee.

Collection and Storage of Blood Sample

5 ml of blood was collected from the patients immediately after admission. Samples were also collected from the controls, under aseptic precautionary measures by using disposable syringe. Out of this 1 ml of whole blood was used for estimation of malondialdehyde and 1 ml was used for the preparation of hemolysate. Enzymes like glutathione reductase and superoxide dismutase were analysed from the hemolysate within one hour.

Methods of Assay

Whole blood:

Malondialdehyde (Thiobarbituric acid method)

Hemolysate:

- Haemoglobin (Drabkin's method)
- Enzymatic antioxidants
 - Superoxide dismutase (Misra and Fridorich)
 - Glutathione reductase (Beutler's E method)

Estimation of Malondialdehyde (MDA) in whole blood⁵

Principle: the reaction depends on the formation of pink coloured complex between malondialdehyde and thiobarbituric acid (TBA), having an absorption of maximum at 532 nm.

Thiobarbituric acid reagent: it is prepared by mixing 75 ml of thiobarbituric acid, 15 g of Trichloroacetic acid and 2.08 ml of 0.2 N HCl. All were mixed and volume made up to 100 ml with distilled water (D/W). All the reagents used were of “analar” grade.

Procedure: two tubes are set up for Blank and Test. Pipette out 0.75 ml of D/W and 3 ml of thiobarbituric acid reagent in “Blank” tube and 0.75 ml whole blood and 3 ml thiobarbituric acid reagent in “Test” tube.

Keep in boiling water bath for 15 minutes. Cool, centrifuge for 10 minutes at 10,000 r.p.m. and read absorbance of supernatants of blank and test immediately at 535 nm.

Calculation

Malondialdehyde (nano moles/ml)

$$\begin{aligned}
 &= \frac{\text{Absorbance of test} \times \text{total volume}}{\text{Nanomolar extinction coefficient} \times \text{Sample volume} \times 100} \\
 &= \frac{\text{Absorbance of test} \times 3.75}{1.56 \times 10^5 \times 0.75 \times 100} \\
 &= \frac{\text{Absorbance of test} \times 3205}{100}
 \end{aligned}$$

Preparation of (RBC) Hemolysate**Isolation of Red Blood Cells⁶**

Most of the enzyme activities in red cells are lower than those in white blood cells (WBCs) and platelets, and hence it was of extreme importance to remove virtually all platelets and WBCs. In order to isolate red blood cells (RBCs) the whole blood was filtered through a column of α -cellulose and microcrystalline cellulose mixture.

α -cellulose and microcrystalline cellulose in 1:1 (w/w) was mixed with isotonic (9.0 g/L) sodium chloride solution. 5 ml plastic disposable syringe without barrel was taken. It was placed in vertical position with the outlet pointing down-

wards. A small piece of filter paper was placed at the bottom of the syringe. The well-mixed cellulose slurry was poured to up to the 2-ml mark. The bed was washed with 5-ml isotonic sodium chloride and 1 ml of whole blood was allowed to flow through the column. To ensure efficient removal of WBCs and platelets, the volume of cellulose mixture used was at least twice as that of the blood sample.

The effluent was collected into a centrifuge tube. The saline suspended red cells were washed twice in at least 10 volumes of ice-cold isotonic sodium chloride. After washing, the packed cells were resuspended in isotonic sodium chloride to give an approximately 50% suspension (1:1 dilution). This suspension was subjected to hemolysis.

Lysing of the RBCs⁷**Reagents**

Stabilizing solution – 2.7 mM ethylene diamine tetraacetic acid (EDTA) (pH 7.0) and 0.7 mM β -mercaptoethanol: this solution was prepared by dissolving 100 mg of disodium salt of EDTA in distilled water and 5 μ l of β -mercaptoethanol (Merck India, Ltd. Mumbai,) were added to it. Final volume was made to 100 ml with D/W.

Procedure

In order to prepare the hemolysate, 1 volume of the RBC suspension was mixed with 9 volumes of the stabilizing solution. The hemolysate was frozen rapidly at -20°C to -25°C in a freezer. Then it was thawed in a water bath at 20°C to 25°C . This hemolysate was then ready for the assay. The hemoglobin estimation was performed on hemolysate using Drabkin's reagent, in order to express the enzyme activities per gram of hemoglobin of the hemolysate. The red cell suspension and its hemolysate were prepared on the day of the assay.

The hemolysate was used for the following enzyme activities: (1) superoxide dismutase (SOD); (2) glutathione reductase (GR).

Determination of Hemoglobin⁸**Principle**

Drabkin's reagent contains potassium cyanide and potassium ferricyanide. Hemoglobin reacts

with ferricyanide to form methemoglobin, which is converted to stable cyanmethemoglobin (HiCN) by the cyanide. The intensity of the colour is proportional to hemoglobin concentration and is compared with a known cyanometemoglobin standard at 540 nm (green filter).

Reagents

1. *Drabkin's reagent*: the prepared reagent was purchased (Span Diagnostics, Surat, India).
2. *Cyanmethemoglobin standard (cyanmeth-Hb standard) 15 g%*: the prepared standard was purchased (Span Diagnostics, Surat, India).

Procedure

Set up 3 tubes, one each for Blank, Standard and Test. Pipette out 5 ml of Drabkin's reagent in Blank, 5 ml of Cyanmethemoglobin standard in "Standard" and both 5 ml of Drabkin's reagent and 0.02 ml of sample (blood). The contents in the tubes were mixed thoroughly and optical density of test and standard (15 g %) were measured at 540 nm against blank (Drabkin's reagent)

Calculation

$$\text{Hemoglobin g \%} = \frac{\text{O.D. test}}{\text{O.D. std}} \times 15$$

Performance of the Enzyme Assays from 1:20 Hemolysate

Superoxide Dismutase⁹

Principle: Epinephrine can be auto-oxidized to adrenochrome by superoxide radicals. Maximum auto-oxidation of epinephrine takes place at pH-10.2. The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine to adrenochrome at pH 10.2 has been used as the basis for the assay of this enzyme. In this method epinephrine acts both as the source of superoxide radical ($\text{O}_2^{\cdot-}$) and as the detecting system giving adrenochrome which can be monitored at 480 nm.

Reagents: (1) Chloroform (2) Ethanol (3) Sodium carbonate/bicarbonate buffer. 0.05 M, pH (10.2). The carbonate buffer was prepared as follows.

- Sodium carbonate (Na_2CO_3) 0.2 M: 2.12 g of anhydrous Na_2CO_3 was dissolved in small quantity of D/W and volume made to 100 ml with D/W.

- Sodium bicarbonate (NaHCO_3), 0.2 M: 1.68 g of NaHCO_3 were dissolved in small quantity of D/W and volume was made to 100 ml with D/W.

Solution (a) 33 ml was mixed with solution (b) 17 ml in small quantity of D/W, pH of the solution was adjusted to 10.2 and volume was made to 200 ml with D/W.

- DL-(α) epinephrine, 3×10^{-2} M: 55 mg of DL-(α) epinephrine were dissolved in 3 ml of D/W. To dissolve epinephrine completely minimum amount of 1NHCl was added and volume was made to 10 ml with D/W.
- EDTA (Na_2EDTA), 15×10^{-3} M: 49 mg of Na_2EDTA were dissolved in minimum quantity of D/W and volume was made to 10ml with D/W.

Procedure: Preparation of hemoglobin free filtrates:

Chloroform, ethanol and water were chilled before use. In a test tube, chloroform (0.125 ml), ethanol (0.25 ml) and D/W (0.8 ml) were added. To this mixture, 0.2 ml of cold hemolysate was transferred. The suspension was subjected to vortex agitation for 2 minutes by transferring the tube intermittently to ice bath. The precipitate was separated by centrifugation at 15,000 rpm at 4°C for 10 minutes and the supernatant was used for the assay.

SOD enzyme assay: For the assay the final reaction mixture in control-contained epinephrine (3×10^{-4} M), EDTA (1×10^{-4} M) and carbonate buffer (0.05 M). In a series of tests, in addition to the above reagents, varying amounts of chloroform-ethanol extracts were added. Table I shows the set up of tubes for SOD enzyme assay.

To control tube 0.03 ml of epinephrine (3×10^{-2} M) was added and after 90 secs (the lag phase) the auto-oxidation of epinephrine was assayed by measuring the optical densities at 0 minute and after 3 minutes, at 480 nm.

To Test₁ 0.03 ml of epinephrine was added and after 90 seconds, optical densities were measured at 0 minute and after 3 minute at 480 nm.

In a similar fashion the assay was performed by adding 0.03 ml of epinephrine one by one to each tube and after lag phase of 90 seconds optical densities were measured at 0 minute and after 3 minutes at 480 nm.

Calculations: SOD activity was determined in terms of its inhibition of auto oxidation of epinephrine to adrenochrome. One enzyme unit is

Table I. SOD enzyme assay- setup of tubes for control and tests.

Reagents	Control	Test ₁	Test ₂	Test ₃	Test ₄	Test ₅	Test ₆
Carbonate buffer, 0.05M pH(10.2) (ml)	2.95	2.90	2.85	2.80	2.75	2.70	2.65
Chloroform-ethanol extract (µl)	–	50	150	150	200	250	300
EDTA, 15 × 10 ⁻³ M (ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02

the amount of protein required to inhibit the auto-oxidation of epinephrine by 50% under standard conditions of assay.

Percent inhibition was calculated from the optical density (OD) of each tube with varying amounts of enzyme (red cell extract) and optical density of the control without enzyme.

$$\text{Percent inhibition} = \frac{100 - \Delta \text{O.Ds} \times 100}{\Delta \text{O.Dc}}$$

Where $\Delta \text{O.Ds}$: Difference in optical density of sample in 3 minutes

$\Delta \text{O.Dc}$: Difference in optical density of control in 3 minutes

A graph percent inhibition against amount of red cell extract was plotted as shown in the figure 16, to determine 50% inhibition of epinephrine oxidation. The amount of extract, which gave

50% inhibition, as determined from the graph corresponds to 1 unit of SOD activity.

$$V \mu\text{l} = 1 \text{ unit of SOD activity}$$

$$\frac{\text{Units of SOD activity} \times 100 \text{ ml of red cell extract}}{X \text{ g of Hb}} = \frac{100 \times 1000 \text{ units}}{V \mu\text{l}}$$

$$\frac{\text{Units of SOD activity}}{\text{g of hemoglobin}} = \frac{100 \times 1000 \times 1}{V \mu\text{l}}$$

Figure 1 shows the graph to be plotted to determine the 50% inhibition of epinephrine. For each sample separate graph has to be plotted.

Calculation: The number of enzyme units per ml:

$$A = \frac{\Delta \text{O.D} \times V_c}{\epsilon \times N \times V_H}$$

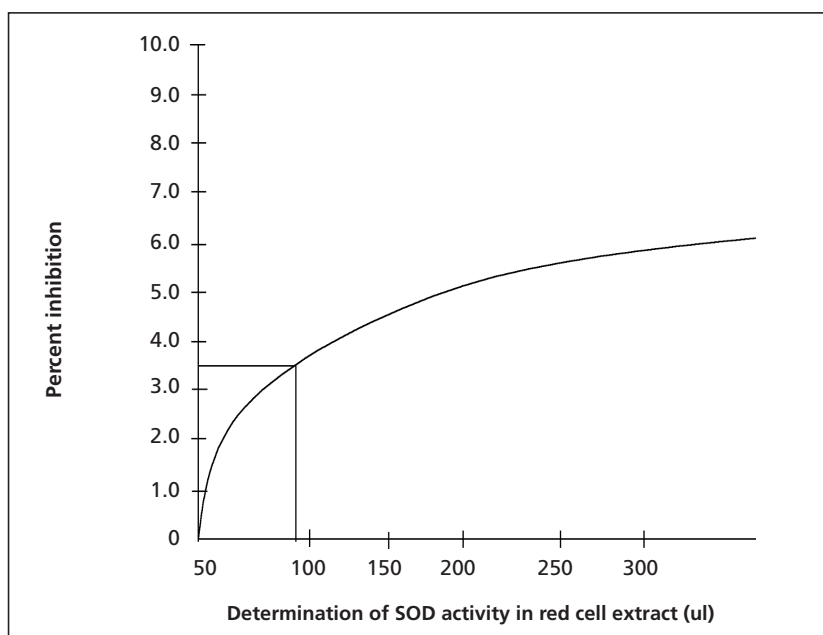


Figure 1. Graph for determination of 50% inhibition of epinephrine.

where, Δ O.D:
change in optical density per minute =

$$\Delta \text{ O.D: } \frac{\text{test} - (\text{Blank}_1 + \text{Blank})}{10 \text{ minutes}}$$

- V_c : the volume of the cuvette in ml = 1 ml
 ϵ : the millimolar extinction coefficient of the nicotinamide adenine dinucleotide phosphate reduced (NADPH) = 6.22
 N : the number of molecules¹ of NADPH converted per molecule of t-Butyl Hydroxy Peroxide (t-BHP) consumed.
 V_H : the volume of hemolysate added to the cuvette in ml = 0.01 ml

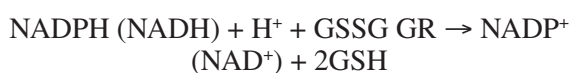
The enzyme activity in international unit/g Hb

$$E = \frac{A \times 100}{\text{Hb}}$$

- A : the number of enzyme units/ml
 Hb : the grams of hemoglobin per 100 ml of the hemolysate.

Glutathione reductase¹⁰

Principle: Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) by NADPH to reduced glutathione.



The activity of this enzyme is measured by following the oxidation of NADPH (NADH) spectrophotometrically (Biomate-6, UV-VIS, Spectrophotometer, Madison, WI, USA) at 340 nm. Glutathione reductase is a flavin enzyme and it has been found that it is not fully activated by flavin adenine dinucleotide (FAD) in normal hemolysates. Complete activation of apoenzyme, requires the preincubation of the enzyme with FAD. This must be done before GSSG or NADPH is added to the reaction system, since these seem to interfere with activation of the enzyme by FAD.

Reagents: (1) Tris-HCl; 1 M, EDTA: 5 mM, pH 8.0: It was prepared as described earlier. (2) Flavin adenine dinucleotide (FAD), 10 μM : 0.8 mg of sodium salt of FAD (SRL, Mwt-829.52) was dissolved in 100 ml of D/W. (3) Glutathione oxidized (GSSG), 0.033M: 20.2 mg of GSSG

(SRL, Mwt-612.7) were dissolved in 1 ml of D/W. (4) Nicotinamide adenine dinucleotide phosphate reduced (NADPH) 2 mM: It was prepared as described earlier.

Table II shows the procedure and set up of tubes for of glutathione reductase assay.

The decrease of optical density was measured at 340 nm at 37°C of the test against the blank.

Calculations: The number of enzyme units per ml:

$$A = \frac{\Delta \text{ O.D} \times V_c}{\epsilon \times N \times V_H}$$

Where

Δ O.D : Change in optical density per minute

V_c : The volume of the cuvette in ml = 1 ml

ϵ : The millimolar extinction coefficient of the NADPH = 6.22

N : The number of molecules¹ of NADPH converted per molecule of GSSG consumed.

V_H : The volume of hemolysate added to the cuvette in ml = 0.01 ml

The enzyme activity in international units/g Hb

$$E = \frac{A \times 100}{\text{Hb}}$$

A : The number of enzyme units/ml

Hb : The grams of hemoglobin per 100 ml of the Hemolysate.

Statistics: Results are expressed as means \pm SD. Statistical analysis was carried out by using Students *t* test (unpaired).

Table II. Glutathione reductase enzyme assay.

	Blank (μl)	Test (μl)
Tris HCl; 1M EDTA, 5 mM, pH 8.0	50	50
1:20 hemolysate	10	10
D/W	790	690
FAD 10 μM	100	100
Tubes were incubated at 37°C for 10 minutes		
GSSG, 0.033M	–	100
Tubes were incubated at 37°C for 10 minutes		
NADPH, 2 Mm	50	50

The decrease of optical density was measured at 340 nm at 37°C of the test against the blank.

Results

Table III shows the concentrations of malondialdehyde and enzymatic antioxidant enzymes in controls and cases.

The present study comprises of 40 clinically diagnosed cases of rheumatoid arthritis and 40 normal healthy controls. The age group ranges from 40 to 60 years.

The mean level of MDA(malondialdehyde) in controls was 6.19 ± 0.96 nmol/ml. The value in cases was 11.48 ± 0.76 nmol/ml. The level was significantly increased ($P < 0.0001$) in RA patients as compared to controls.

The value of SOD (superoxide dismutase) in controls was 948.32 ± 99.88 IU/g of Hb. The mean value of SOD was 443.68 ± 111.69 IU/g Hb in the total cases. The level of SOD was significantly decreased ($P < 0.0001$) in cases compared to controls.

The mean glutathione reductase level was 8.91 ± 1.04 IU/g of Hb in controls and the level in cases was 2.96 ± 0.79 IU/g of Hb. The level of glutathione reductase was decreased significantly ($P < 0.0001$) in all the cases as compared to controls.

Discussion

Rheumatoid arthritis is a major cause of morbidity as it affects the joints, causing stiffness and loss of mobility. The cause of rheumatoid arthritis is mainly joint inflammation initiated by oxidative stress.

Involvement of oxygen free radicals (OFR) in the pathophysiology of inflammation in a number of organs and tissues has been reported in literature^{11,12}. Evidence of OFR generation in patients with RA has been observed by measuring

the product of lipid peroxidation malondialdehyde. Antioxidant status was assessed by measuring superoxide dismutase and glutathione reductase, In view of the recent animal studies strongly suggesting anti-inflammatory role of antioxidants like superoxide dismutase¹³ and vitamin E¹⁴ in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA¹⁵⁻²¹. Hypoxic conditions also disrupt an intracellular ionic environment and alter calcium and phosphorous levels. So, estimation of calcium and phosphorous levels was done.

In the present study mean level of MDA was increased significantly in cases compared to controls. Our findings are in accordance with the research of Shaabani et al²², Walwadkar et al²³, Ansari and Jaiswal²⁴. MDA is a decomposition product of lipid peroxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. Gambhir et al²⁵ also reported markedly increased concentrations of MDA in patients as compared to controls. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process. Similar reports of elevated MDA levels have been reported in patients with rheumatoid arthritis²⁶.

The mean level of SOD was significantly decreased in cases compared to controls ($P < 0.0001$). The findings of our study are in accordance with study of Bae et al²⁷.

DiSilvestro et al²⁸ showed that the administration of anti-inflammatory drugs increases plasma SOD activity, indicating the inflammation process produces free radicals, thereby decreasing SOD activity. Disease itself may inhibit the activity of SOD and reduce the synthesis of SOD.

Our findings are contradictory to the findings of Surapneni and Gopan²⁹ who showed significant increase in SOD levels in RA.

Table III. MDA and Enzymatic Antioxidants in controls and cases.

Parameters	MDA nmol/ml	SOD (IU/g of Hb)	Glut red (IU/g of Hb)
n = 40 (M-20, F-20) Controls Mean + S.D	6.19 + 0.96	948.32 + 99.88	8.91 + 1.04
n = 40 (M-20, F-20) Cases Mean + S.D	11.48 + 0.76	443.68 + 111.69	2.96 + 0.79
P. value	< 0.0001	< 0.0001	< 0.0001

The mean level of glutathione reductase was decreased significantly in cases ($P < 0.0001$) compared to controls.

The findings of the present study are in accordance with the results of Palanisamy Pasupathil et al³⁰, who have observed an enhanced lipid peroxidation in rheumatoid arthritis patients with a concomitant failure of both the plasma, and erythrocyte antioxidants defense mechanism. These results are consistent with the underlying hypothesis that there is an imbalance between ROS production and the antioxidant defense system in inflammatory RA disease.

Conclusion

In RA there is an increased oxidative stress and decreased enzymatic antioxidant defense which is the root cause of joint inflammation causing arthritis.

References

- 1) FAUCI AS, BRAUNWALD E, KASPER DL, HAUSER SL, LONGO DL, JAMESON JL, LOSCALZO J. Harrison's Principles of Internal Medicine. 17th Edition, McGraw Hill, New York, pp. 2083-2092, 2008.
- 2) DUTHIE RB, BENTLEY G, DHAL AK. Mercer's Orthopaedic Surgery, 9 th Edition, Jaypee Brothers Medical Publishers(P) Ltd, New Delhi, pp. 754-774, 2003.
- 3) DM VASUDEVAN, SREEKUMARI. Text book of biochemistry, 4th edition.p:234-35, Publisher: Jaypee Brothers, New Delhi, 2005.
- 4) COX MM, NELSON DL. Lehninger Principles of Biochemistry. 5th Edition, p. 259, W.H.Freeman & Company, New York, 2008.
- 5) PLACER ZA, LINDA L, CRUSHMAN JBC. Estimation of product of lipid peroxidation in biochemical system. Annal Biochem 1966; 16: 359-364.
- 6) BEUTLER E, WEST C, BLUME KG. The removal of leucocytes and platelets from whole blood. J Lab Clin Med 1976; 88: 328-333.
- 7) BEUTLER E, BLUME KG, KAPLAN JC, LOHR GW, RAMOT B, VALENTINE WN. International Committee for Standardization in Haematology; Recommended methods for red-cell enzyme analysis. Brit J Haematol 1977; 35: 331-340.
- 8) DRABKIN DL, AUSTIN JH. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 1932; 98: 719-733.
- 9) MISRA HP, FRIDORICH I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170-3175.
- 10) BEUTLER E. Red cell metabolism. A manual of biochemical methods. London Edinburg Churchhill Livingstone Publication, Vol-16, 1986.
- 11) FREI B, STOCKER R, AMES BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci USA 1988; 85: 9748-9752.
- 12) UHLING S, WENDEL A. The physiological consequences of glutathione variations. Life Sci 1992; 51: 1083-1094
- 13) SALVEMINI D, MAZZON E, DUGO L, SERRAINO I, DE SARRO A, CAPUTI AP, CUZZOCREA S. Amelioration of joint disease in a rat model of collagen induced arthritis by M40403, a superoxide dismutase mimetic. Arthritis Rheum 2001; 44: 2909-2921.
- 14) BEHASKA AA, WU D, SERAFINI, MEYDANI-SN. Mechanism of vitamin E inhibition of cyclooxygenase activity in macrophage from old mice: role of peroxynitrite. Free Radic Biol Med 2002; 32: 503-511.
- 15) DE BANDT M, GROSSIN M, DRISS F, PINCEMAIL J, BABIN-CHEVAYE C, PASQUIER C. Vitamin E uncouples joint destruction and clinical inflammation in a transgenic mouse model of rheumatoid arthritis. Arthritis Rheum 2002; 46: 522-532.
- 16) HELMY M, SHOHAYEB M, HELMY MH, EL-BASSIOUNI EA. Antioxidants as adjuvant therapy in rheumatoid disease. A preliminary study. Arzneimittel-Forschung 2001; 51: 293-298.
- 17) JASWAL S, MEHTA HC, SOOD AK, KAUR J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clin Chim Acta 2003; 338: 123-129.
- 18) DARLINGTON LG, STONE TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Br J Nutr 2001; 85: 251-269.
- 19) HAGFORS L, LEANDERSON P, SKÖLDSTAM L, ANDERSSON J, JOHANSSON G. Antioxidant intake, plasma antioxidants and oxidative stress in a randomized, controlled, parallel, Mediterranean dietary intervention study on patients with rheumatoid arthritis. Nutr J 2003; 2: 5.
- 20) BAE SC, KIM SJ, SUNG MK. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients. J Am Coll Nutr 2003; 22: 311-315.
- 21) CERHAN JR, SAGG, KG, MERLINO, MIKULS TR, CRISWELL LA. Antioxidant micronutrients and risk of rheumatoid arthritis in a cohort of older women. Am J Epidemiol 2003; 157: 345-354.
- 22) SHAABANI Y, FOROUGHI M, RASTMANESH R, JAMSHIDI A, TAJIK N, ASSADI O. Assessment of antioxidant nutrient intake and malondialdehyde plasma level in rheumatoid arthritis. Atherosclerosis J 2009; 5: 1-5.

- 23) WALWADKAR SD, SURYAKAR AN, KATKAM RV, KUMBAR KM, ANKUSH RD. Oxidative stress and calcium phosphorous levels in rheumatoid arthritis. *Indian J Clin Biochem* 2006; 21: 134-137.
- 24) ANSARI S, JAISWAL G. Oxidative status in rheumatoid arthritis—A biomedical study. *Antiseptic* 2008; 105: 429-431.
- 25) GAMBHIR JK, LALI P, JAIN AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. *Clin Biochem* 1997; 35(1-4): 89-98.
- 26) KARTAS F, OZATES, CANATAN H, HALIFEGLUE I, KARATAPE M. Colakt. Antioxidant status and lipid peroxidation in patients with rheumatoid arthritis. *Ind J Med Res* 2003; 118: 178-181.
- 27) BAE SC, KIM SJ, SUNG MK. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients. *J Am Coll Nutr* 2003; 22: 311-315.
- 28) DI SILVESTRO RA, MARTEN J, SKEHAN M. Effects of copper supplementation on ceruloplasmin and Cu/Zn superoxide dismutase in free living rheumatoid arthritis patients. *J Am Coll Nutr* 1992; 11: 177-180.
- 29) SURAPNENI KM, CHANDRASADA GOPAN VS. Lipid peroxidation in patients with rheumatoid arthritis. *Indian J Clin Biochem* 2006; 23: 41-44.
- 30) PALANISAMY PASHUPATHIL P, MATHIYALAGAN DEEPA M, RANI P, RAMESH SHANKAR R. Circulating lipid peroxidation, plasma and erythrocyte antioxidant status in patients with rheumatoid arthritis. *Bangladesh Med Res Council Bull* 2009; 35: 57-62.

This review article shows the relation between oxidative stress and role of antioxidant in osteoarthritis and rheumatoid arthritis. Arthritis is a generic term used for any of over hundred diseases of the joints which are accompanied by pain, stiffness and swelling. Many individual get crippled by arthritis, Arthritis thus refers to the pain and inflammation of the joints. The two most common and best known types of arthritis are Osteoarthritis, a wearing away of the cushioning cartilage in the joints: and Rheumatoid arthritis, an autoimmune disease that causes painful and often disabling joint inflammations. Free radicals

CONTINUE READING. 2 Citations.

Fields of Study. Fields of Study. Oxidative stress and decreased antioxidant status are the hallmarks in patients of RA as observed in recent years. The objectives of the study was to determine oxidative stress by measuring malondialdehyde and enzymatic antioxidant status by estimating superoxide dismutase and glutathione reductase in patients of RA and then comparing with healthy individuals.

Results: The mean level of blood malondialdehyde (in nmol/ml) in controls was 6.19 +/- 0.96 and 11.48 +/- 0.76 in cases. The mean level of superoxide dismutase (IU/g of Hb) in controls was 948.32 +/- 99.88 and 443.68 +/- 111.69 in the cases.

Conclusions: This study revealed that there was an increased oxidative stress and a decreased antioxidant defense in patients with rheumatoid arthritis. The study of oxidants and antioxidants biomarkers in RA patients could improve our understanding of disease pathogenesis; likely determining the oxidative stress levels in these patients could prove helpful in assessing disease activity and might also have prognostic implications. To date, the usefulness of oxidative stress biomarkers in RA patients is unclear and the evidence supporting them is heterogeneous.

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease whose pathogenic mechanisms remain to be elucidated. The oxidative stress and antioxidants play an important role in the disease process of RA. Oxidation in rheumatoid arthritis.

Carol A Hitchon¹ & Hani S El-Gabalawy. Oxidative stress is a term that is used to describe situations in which the organism's production of oxidants exceeds the capacity to neutralize them. The result can be damage to cell membranes, lipids, nucleic acids, proteins, and constituents of the extracellular matrix such as proteoglycans and collagens.

In this review we explore the role of oxidative stress and hypoxia in the pathogenesis of rheumatoid arthritis (RA), a prototypical chronic inflammatory disorder, focusing on recent developments in this area, and highlighting mechanisms that can potentially be exploited therapeutically.

Thioredoxin regulates protein redox status and, when activated, facilitates protein-DNA interactions. Joint destruction in rheumatoid arthritis (RA) is due to tissue injury in the area caused by inflammatory reactions, release of MMPs and free radicals produced by neutrophils and macrophages. The control of free radical production may have therapeutic roles thus the study was done to check the status of lipid peroxidation product malondialdehyde (MDA) and a few antioxidant enzymes in RA patients.

Oxidative stress occurs due to disturbed balance between body's antioxidant mechanisms and oxidative stress production and has important role in the development of chronic disease as autoimmunity like RA, cancer etc. [5-7]. However, studies [11,12] show that enzymatic/non enzymatic antioxidant systems are highly deregulated and impaired in RA.