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Study of Lipid per Oxidation and Enzymatic Antioxidant Activity in Rheumatoid Arthritis: Relation with Disease Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Rheumatoid Arthritis (RA) is one of the known Autoimmune diseases in patients with age group of 25 to 60. A significant aspect of disease development should be Reactive Oxygen Species (ROS). Few studies have shown an association between oxidative stress and RA disease activity. This study was therefore intended to examine whether there is a link between oxidative stress and disease activity.
Methods and Materials: This case-control study on 120 individuals was conducted. In accordance with the Disease Activity Score (30) were healthy controls with 90 RA patients in mild (30), moderate (30) and severe (30). Malondialdehyde Measured Lipid Per Oxidation (MDA). Antioxidant enzyme activities such as Superoxide Dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPX) have been also measured. A correlation coefficient was also used to evaluate the strength of the link between oxidative stress and activity of the illness.
Results: the MDA levels in all the RA subgroups were substantially higher than those in the control group, but the activity of antioxidant enzymes was significantly lower. Between Malondialdehyde

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Measured Lipid Per Oxidation (MDA) and Disease Activity score of 28 RA patients (DAS28) there were positive correlations.
Conclusion: Significant increase in RA patients' lipid per oxidization supports the role of oxidative stress in RA aetiology and the usage of MDA for disease control.

Keywords: Rheumatoid arthritis; lipid peroxidation; antioxidants; glutathione peroxidise; DAS28.

1. INTRODUCTION

Peripheral, symmetric polyarthritis-characterized disorder leads to damage in joint and physical impairment [1]. The global prevalence of RA is around 1-1,5% of the population [2]. While RA is a non-fatal condition, greater mortality is overall associated with the diseases. In RA patients, the mortality rate in comparison to general population is around 1.5 times greater [3].

Unknown is the aetiopathogenesis of RA, although some investigations have revealed a union of the genetic and environmental variables leading to immunosuppressive defects and inflammatory mechanisms, including oxidative stress [4]. The autoimmune disease of rheumatoid arthritis affects the cells of the body's immune system. The immune system's highly multiplying cells have a strong risk of free radical damage. Number of cells in the joints can produce ROS, causing oxidative stress and producing nitric oxides. Oxidative stress arises when production of oxidants beat the efficiency of antioxidants to knock out the oxidant products, which promote consequent injury to cells and tissues. It appears to be an increase in damage to various biomolecules. Different tissues are differently vulnerable to oxidative stress. Synovial membrane is more susceptible to oxidative damage due to the formation of 'pannus' and accumulation of inflammatory cells which produces ROS via 'respiratory burst' [5].

The harmful toxic effect caused by ROS is counteracted by cell's antioxidant defence system to minimize the damage. Antioxidants are extrinsic or intrinsic in nature. They either inhibit the synthesis of toxic oxidants or arrest those generated and inactivate them and intercept the multiplication of chain reaction initiated by these oxidants [6]. This protective mechanism works cooperatively, which include various antioxidants like catalase, superoxide dismutase and glutathione peroxidase that are enzymatic and some other molecules like vitamin C, E, reduced glutathione, uric acid, etc.

Now a day, there is an increase in prevalence of rheumatoid arthritis with the increase in urbanization and lifestyle. The global RA prevalence estimate was 0.46% (95% confidence interval [CI] 0.39-0.54; I^2 = 99.9%) with a 95% prediction interval (0.06-1.27). The RA point-prevalence was 0.45% (95% CI 0.38-0.53%) between 1986 and 2014, while the pooled period-prevalence was 0.46% (95% CI 0.36% and 0.57%) from 1955 to 2015 [7]. There are many causative factors such as oxidative stress and inflammatory markers of RA. While going through different research articles, it was observed that there is a discrepancy in most of the results regarding all these parameters. Here there is intended to understand the pathogenesis, behind RA in terms of oxidative stress to study the balancing components which are present in the body in the form of antioxidants and to evaluate their correlation.

2. METHODS

The study was done at SMBT Dental College, Sangamner and the patients were the in and out patient department from nearby hospitals. The Ethical Committee of the institute approved this work.

2.1 Inclusion Criteria

The patients with Disease Activity score of 28 (DAS 28) the criteria of RA were enrolled in study [8]. In the present study, total 120 subjects were included; from this 30 were control and 90 were Rheumatoid arthritis patients with age between 25 to 60 years. Based on the Activity Score they were categorized into [9] mild, moderate and severe each with 30 patients at the time of visit to hospital.

2.2 Exclusion Criteria

Patients who were smokers, alcoholics and had previous or coexisting diseases like anaemia, any other inflammatory joint diseases, connective tissue diseases, diabetes mellitus, heart and renal diseases, infectious or allergic diseases that may affect redox status were not included in the study.

2.3 Laboratory Analysis

With prior consent from subject, 5 ml of blood was drawn from which 3 ml was taken into plain bulb and 2 ml into heparin bulb. Serum obtained was used for estimation of Malondialdehyde Measured Lipid Per Oxidation (MDA) and for measurement of activity of Catalase (CAT) [10] and erythrocyte- Superoxide Dismutase (SOD) [11].

2.4 Statistics

Results obtained were expressed in terms of Mean ± SD. Values were analysed statistically by using Software Package for the Social Sciences. P < 0.01 was treated as significant [12]. The power of the interrelation within the variables was measured by calculating Pearson's Correlation Coefficient (r value).

3. RESULTS

Table 1 Shows the demographic characteristics of healthy controls and RA patients. There is no remarkable variation between age of the control group and patients.

Free radicals attack cell components including lipids resulting in production of MDA, which is used as a biomarker of lipid per oxidation. In our study, we found MDA raised in mild, moderate and severe RA patients in comparison to control group (Table 2). MDA plasma levels correlate closely with plasma 8-isoPGF$_{2\alpha}$ levels [13]. Further, we observed that as the severity of the disease increases, the level of MDA increases significantly. It indicates that the extent of MDA elevation is associated with the disease progression [14]. Table 2 shows the comparison between lipid per oxidation marker (MDA) and antioxidant enzymes (SOD, CAT, GPX) in controls and RA patients. MDA exhibits high reactivity and ability to form adducts with many biological molecules, the majority of MDA is bound to DNA and amino acid moieties in proteins [15].

Mammalian cells developed an antioxidant system to counteract on ROS generated products. It contains enzymatic and non-enzymatic antioxidants.

In comparison with healthy controls, the values of erythrocyte-SOD, serum CAT and blood GPX in mild, moderate and severe RA patients were decreased. Furthermore, erythrocyte-SOD, serum CAT and blood GPX activities in moderate RA patients was lowered significantly when compared with mild RA while RA patients having severe disease activity presented with significant (P< 0.01) decrease in erythrocyte-SOD, serum CAT and blood GPX activity in comparison with moderate RA patients [16]. Results from the correlation analysis are summarised in Table 3.

Table 1. The healthy subjects are displayed

Parameter	Control	Rheumatoid Arthritis		
		Mild	Moderate	Severe
Age in years	37.16 ± 7.46	37.8 ± 5.50	38.36 ± 7.11	37.66 ± 7.44
Gender (F/M)	13/17	18/12	16/14	20/10
ESR (mm/Hr)	14.06 ± 3.38	27.5 ± 5.5	45.16 ± 4.63	62.03 ± 4.41
CRP (mg/L)	8.78 ± 2.64	22.49 ± 4.39	29.83 ± 5.03	35.67 ± 4.64.
DAS28	NA	2.73 ± 0.35	3.89 ± 0.44	5.87 ± 0.39

Table 2. The comparison study between lipid peroxidation marker (MDA) and antioxidant enzymes (SOD, CAT, GPX) in controls and RA patients

Parameter	Control	Rheumatoid Arthritis		
		Mild	Moderate	Severe
MDA (nmol/ml)	1.64 ± 0.34	2.83 ± 0.45*	3.33 ± 0.44*•	3.94 ± 0.39*≠
SOD (U/mg of Hb)	3.57 ± 0.68	3.03 ± 0.56*	2.51 ± 0.53*•	2.09 ± 0.56*≠
CAT (KU/L)	60.72 ± 10.46	55.04 ± 7.29*	52.71 ± 6.43*•	49. 63 ± 7.61*≠
GPX (U / g of Hb)	20.16 ± 2.59	16.51 ± 2.79*	14. 18 ± 2.91*•	11.91 ± 2.12*≠

** P<0.01 healthy control*
●P<0.01 mild RA patients
≠P<0.01 moderate RA patients
SOD - Superoxide Dismutase
CAT - Catalase
GPX - Glutathione peroxidase

Table 3. Correlation between MDA, antioxidant enzymes and DAS 28

	Mild RA (DAS28< 3.2)	Moderate (DAS28 between 3.2-5.1)	Severe (DAS28> 5.1)
MDA Vs SOD	-0.98	-0.95	-0.93
MDAVs CAT	-0.97	-0.96	-0.97
MDA Vs GPX	-0.94	-0.97	-0.95
MDA Vs DAS 28	0.93	0.87	0.77

Table 3 shows significantly negative correlation between MDA and antioxidant enzymes in mild, moderate and severe RA patients. It also shows score i.e. DAS28 in all the three grades of RA disease. i.e. mild, moderate and severe.

4. DISCUSSION

Rheumatoid arthritis can be a severe crippling disorder. Despite the various extensive studies, the aetiopathogenesis of RA remains unclear. The RA pathogenesis is multifactorial, with recently suggested involvement of active oxidative processes [17].

Various reports have explained the stimulation of the production of ROS and subsequent activation of inflammatory molecules involved in RA progression.

ROS carry out the degradation of hyaluronic acid which leads to decrease in viscosity of synovial fluid. ROS produced by phagocytes from RA joints may not be effectively washed out by antioxidants resulting in elevated levels due to their transportation by circulatory system.

The present study shows significantly increased level of MDA in RA in comparison with control. These findings are in harmony with amplified level of MDA in RA patients [18].

There is arealization that ROS may play a key role in mediating tissue damage and cellular injury (R144). MDA- acetaldehyde adducts (MAA) suggest that MDA causes tolerance loss in pain which leads to autoimmune response.

When we look at the outcome of our study, RA patients was confirmed by significantly positive correlation between MDA and DAS28 in mild, patients. From this we can infer that levels of lipid per oxidation are related to disease activity. From these findings, we can suggest serum MDA could also be used for prognosis of the disease. Determination of MDA levels has historically relied on a reaction with thiobarbituric acid (TBA) to generate the products known as “thiobarbituric acid reactive substances” (TBARS) that can be measured by colorimetry (532 nm) or fluorimetry (excitation at 532 nm and emission at 553 nm). Since the TBARS test is an easy and cost-effective method, it is widely used for routine analysis of MDA in clinical laboratories [19].

Cells defend themselves against free radical attacks by the antioxidants system. This system consists of non enzymatic, low molecular weight, non-protein antioxidants (vitamin E, C, uric acid) and enzymes (SOD, CAT, GPX and glutathione reductase) reactions. Neutrophils act by Superoxide dismutase along with catalase and glutathione peroxidise dependent mechanisms.

The first line of antioxidant defence comprises of SOD which catalyses and convert superoxide anion it to H_2O_2. This H_2O_2 is further detoxified by CAT and GPX system. This transformation of H_2O_2 is important because it prevents formation of toxic compounds like peroxynitrite ($ONOO^-$) and hydroxyl radical (OH^-).

There are controversial reports on erythrocytes-SOD, GSH-Px and CAT activity in RA patients.

We found significant decrease. However, increase in SOD activity and found no change in the activity of SOD enzyme. These discrepancies can include diet, epigenetic factors, age or gender. Decrease in the erythrocyte-SOD may be due to its utilization in the detoxification of superoxide anion ($O_2^{-\bullet}$) or may be due to its inactivation by disease itself. As the natural antioxidant defence system SOD limits the advancement of the inflammatory and immune mechanism and its decreased activity may be responsible for increased disease activity.

One of the antioxidant enzymes which detoxify H_2O_2 is catalase. In comparison with control this activity goes on decreasing as the disease progresses. Lowered catalase activity may be due to its inactivation by H_2O_2. Expression of Catalase alters expression of genes responsible for inflammation. Thus, decreased activity of catalase may be one of the reasons for

increased inflammation in RA. Further, inactivation of SOD may result in increased level of H_2O_2 which may induce CAT inactivation.

5. CONCLUSION

In our study, the activity of GPX in mild, moderate and severe RA patients decreased significantly compared with management. GLP and Catalysis utilise H_2O_2 as a substrate, however catalysis at higher H_2O_2 concentrations and GPX at lower concentration. H_2O_2 is the substrate used.

Knowledge of the interconnections between the different pathways might assist to choose the RA therapeutic strategy. MDA might be utilised as a biochemical marker for the activity of diseases but did not identify any link between the activity of antioxidants and diseases.

The findings show that GPX activity is enhanced whereas Staron does not affect GPX activity. Increased lipid per oxidation and impaired enzyme antioxidant mechanisms enhance oxidative stress in RA development. Lipid per oxidation can be used as a monitoring indicator for the activity of the illness in RA patients. RA may be considered as an alternative for oxidative stress control therapy. Catalase and or glutathione peroxidise may give more protection by antioxidant supplementation. Thus, Catalase-glutathione enzyme supplements can be empirically considered based on the above mentioned data in RA patients.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

Institutional Ethical Committee approved the protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aryaeian N. Effect of conjugated linoleic acids, vitamin E and their combination on the clinical outcome of Iranian adults with active rheumatoid arthritis. International Journal of Rheumatic Diseases. 2009;12:20–28.
2. Ben-Hadj-Mohamed M. A case control study of antioxidant and oxidant status in Tunisian rheumatoid arthritis patients. IJPSR. 2016;7:2591- 2596.
3. Bhowmick K. Free radicals and antioxidant status in rheumatoid arthritis. Indian Journal of Rheumatology. 2008;3:8-12.
4. Baskol G. Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis Cell Biochem Fun; 2005.
5. Das K. A modified spectrophotometric assay of superoxide dismutase using nitrate formation by superoxide radicals. Indian J Biochem and Biophys. 2000;57:201-214.
6. Ajay K S. Study of nonenzymatic antioxidant and lipid peroxide levels in rheumatoid arthritis. Universal Journal of Medicane and Dentistry. 2012;1:10-13.
7. Khalid Almutairi , Johannes Nossent , David Preen , Helen Keen , Charles Inderjeeth. The global prevalence of rheumatoid arthritis: A meta-analysis based on a systematic review. 2021;41(5): 863-877.
8. Alethaha D. Rheumatoid arthritis classification criteria: An American college of rheumatology/European league against rheumat ism collaborative initiative. Arthritis & rheumatism. 2010;62:2569–258.
9. Desai PB. Oxidative stress and enzymatic antioxidant status in rheumatoid arthritis: a case control study. European Review for Medical and Pharmacological Sciences. 2010;14:959-967.
10. Garry TM. Hypoxia, oxidative stress and inflammation. Free Radical Biology and Medicine. 2018;12:15-24.
11. Goth L. A simple method for determination of serum catalase activity and revision of reference range Clinica Chimica Acta. 1991;196:43-152.
12. Guzel S. Nitric oxide and superoxide dismutase in rheumatoid arthritis: Correlation with disease activity. Turkish Journal of Family Medicine and Primary Care (TJFMPC). 2012;6:7-12.
13. Tsikas D, Suchy MT, Niemann J, Tossios P, Schneider Y, Rothmann S, Gutzki FM, Frölich JC, Stichtenoth DO. Glutathione promotes prostaglandin H synthase (cyclooxygenase)-dependent formation of

malondialdehyde and 15(S)-8-iso-prostaglandin F2α FEBS Lett. 2012;586: 3723–3730.
DOI:10.1016/j.febslet.2012.09.001

14. Hilal K. Relationship of cellular oxidant and antioxidant status with disease activity in patients with rheumatoid arthritis. Turk J Rheumatol. 2010;25:141-146.
15. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal. Biochem.* 2017;524:13–30.
16. Hitchon CA. Oxidation in rheumatoid arthritis Arthritis Res ther. 2004;6:265-278
17. Garcia-Gonzalez A Ramon. Oxidative stress in patients with rheumatoid arthritis. Rev Invest Clin. 2015;67:46-53.
18. Hoek J. Mortality in patients with rheumatoid arthritis: a 15-year prospective cohort study Rheumatol Int. 2017;37:487-493.
19. Yu LW, Latriano L, Duncan S, Hartwick RA, Witz G. High-performance liquid chromatography analysis of the thiobarbituric acid adducts of malonaldehyde and trans, trans-muconaldehyde. Anal. Biochem. 1986;156: 326–333.
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