Determination of Malondialdehyde (MDA) and Some Antioxidant Activities in Rheumatoid arthritis Patients

Elham Abdulqadir Abdulrahman[1], Halit Demir[2] and Bakhtyar Kamal Aziz[3,*]

[1] Department of Pharmaceutical Chemistry, College of Medical and Applied Sciences, Charmo University, Chamchamal 46023, Iraq.

[2] Van Yuzuncu Yil University, Faculty of Science, Department of Chemistry, Van, Turkey

[3] Department of Nano-Science and Applied Chemistry, College of Medicals and Applied Sciences, Charmo University, Cahmchamal 46023, Iraq

*Corresponding Author:

Email: bakhtyar.kamal@charmouniversirt.org

ORCID ID : 0000-0003-3348-4884

Abstract

Background: Muscle weakness is a common comorbidity of several severe diseases which results in a reduced independence and quality of life and for the afflicted patients. Patients with rheumatoid arthritis (RA), a disease characterized by a chronic inflammation of the joints, commonly report of muscle weakness as a complication. Muscle weakness is the result of reduced muscle size and intramuscular impairments (i.e., intrinsic muscle dysfunction).

Methods: In this study, patients with muscle and joint weakness were sub-grouped. Thirty-six RA patients (16 males and 20 females) and forty healthy and age matched (control) were evaluated for superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT, and reduced glutathione (GSH) levels using spectrophotometric methods.

Results: This study shows that the oxidative status affects very well the cellular damage of the tissue in RA patients. Rheumatoid arthritis patients showed a higher ROS production, the data showed that the mean level of serum MDA of the patient groups is significantly higher compared to control group. Whereas the mean level of SOD activity of patients group is significantly lower than that of healthy control group in rheumatoid

($p<0.05$). There are significant differences in the mean levels of serum GSH and CAT of patient groups compared to the control groups. This study shows that the oxidative status affects very well the cellular damage of the tissues in RA patients.

Keywords: *inflammation, rheumatoid arthritis, antioxidants, joint weakness*

1. Introduction

Muscle and joint weakness are kind of disease which characterized by a chronic inflammation of the joints and typically known as rheumatoid arthritis. Rheumatoid arthritis is a symmetrical, chronic, inflammatory autoimmune disease that affects small joints at first, and then it spreads to larger joints, and eventually affecting the skin, heart, eyes, kidneys and lungs. Joints typically lose their bone and cartilage, which weakens tendons and ligaments [1]. All this damage that occurs to the joints makes deformities and bone erosion that is typically very painful for the patients. Rheumatoid arthritis (RA) is a disease with an average prevalence of about 1% adults [2]. The disease can strike anyone at any age and affects women 2 to 3 times more often than men. The peak occurrence of this disease is in the sixth decade [3]. In the past, RA was the reason of disability, inability to work, and higher mortality rate. latest improvement in outcomes has been gained through a better understanding of pathophysiology of RA and progression of better outcome measures and medicines. The body damage due to RA is not only limited to joints [4]. In fact, the systemic inflammatory environment noticed in RA patients leads them to increase the risk of cardiovascular [5], besides potential damage to several organs, containing, but not only limited to, the eyes [6], lungs [7], heart [8], small vessels [9], and skin [10] eventually suggesting a larger risk of dying too soon [11].

Oxidative stress is a fundamental player in the irritation of chronic inflammatory joint disease. Both experimental models and estimations in patients revealed, extra to elevated reactive oxygen species (ROS) and lipid formation of peroxidation, a reduction in

antioxidant defenses [12]. The participation of oxidative stress has been demonstrated in the pathogenesis of inflammatory diseases, such as RA [13]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are significant categories of molecules produced in living systems for cellular metabolism. However, when such reactive species exceeds concentrations over the top limit of normal range, they defect cellular components [14]. In this way, therapies that reduce oxidants and/or elevate antioxidants are promising of various oxidative stresses related inflammatory diseases treatment [15]. RA patients are treated with antioxidant vitamins A, E, and C together with formal disease-modifying antirheumatic drugs (DMARDs) for 12 weeks indicated minimized levels of MDA and elevated concentrations of thiols and decreased glutathione (GSH) and vitamin C concentrations in blood of samples [16]. These patients also displayed reduced Rheumatoid Arthritis Disease Activity Index (RADAI), proving that the therapy of antioxidant was effective in improving both the redox profile and the disease activity in these patients [17]. On the other side, the increased consumption of foods rich with antioxidant during 3 months did not change plasma antioxidants and urine MDA levels in RA patients. however, the levels of plasma retinol showed an opposite correlation with the erythrocyte sedimentation rate (ESR), disease activity score (DAS-28), and C-reactive protein (CRP); the vitamin C, a negative association with ESR and the health assessment questionnaire (HAQ) score; the levels of uric acid, in turn, were inversely correlated with the thrombocyte count, pointing to the relationship between levels of serum uric acid and inflammation degree in RA patients [18].

The present study was designed to determine the importance of oxidative stress level and determining some antioxidant status in the blood of RA patients. Malondialdehyde (MDA) level was measured as a marker for oxidative lipid damage because it is the final product of lipid peroxidation. Levels of GSH, SOD, and catalase, were also measured in RA patients and were compared to healthy controls.

2. Materials and Methods

2.1. Study design

This research was conducted in the Department of science, Van Yüzüncü Yıl University. The study was performed with 36 RA patients (16 males and 20 females) [19]. The control group consisted of 40 healthy and age matched individuals (15 males and 25 females). Blood samples were taken from patients and healthy individuals, 4 mL of blood was taken from an antecubital venous vein and 2 mL of the blood was added to the biochemistry tube and the other 2 mL to the EDTA tube.

2.2. Blood samples

Thirty-six patients who were diagnosed with rheumatoid arthritis (RA) and 40 healthy individuals were selected with age ranging between 21-84 years. The blood samples were centrifuged at 45000 rpm for 3 minutes to separate the serums from plasma. The separated serums were used to determine the antioxidants superoxide dismutase (SOD), reduced glutathione (GSH), catalase enzyme activity (CAT) and malondialdehyde (MDA) levels.

The control group consisted of men over 50 years of age, who were eligible for the study if they had not been diagnosed with cancer throughout their lives and they had had no major surgery.

2.3. Determination of superoxide dismutase (SOD)

Superoxide dismutase activity was determined by using the proposed method of Popov et al. SOD accelerates the dismutation of hydrogen peroxide and molecular oxygen of superoxide radicals ($O_2^{-\bullet}$) formed during the oxidative energy production. The mechanism of this method is based on reading the optical density (λ_{max}= 560 nm) of the blue colored dye of the nitro blue tetrazolium (N.B.T) that changes due to the superoxide radicals generated using xanthine and xanthine oxidase. Inhibition of the formation reaction by the SOD that exists in the sample serum by excluding superoxide radicals from the reaction [20].

Under the experimental conditions, 1 unit of SOD is the % 50 inhibition of N.B.T reduction rate.

% Inhibition= [(Blank OD – Sample OD) / Blank OD] x 100

2.4. Determination of Reduced Glutathione (GSH)

From the hemolysis of EDTA blood, a yellow color was obtained by the reaction of the (SH) groups of the reduced glutathione with of 5', 5'-(dithiobis 2-nitrobenzoic acid) (DTNB). The optical density measurements were done at 412 nm wavelength of the spectrophotometer.

Activity (mg/ dL) = [(OD2 – OD1)/ 13600 x 1:25] x 1000

OD1= Initial absorbance before addition of DTNB at 412 nm.

OD2= Absorbance after addition of DTNB at 412 nm.

13600 is the molar extinction coefficient of the yellow color that formed during the interaction of GSH and DTNB.

2.5. Determination of Catalase (CAT) Activity

Hydrogen peroxide was used as a substrate in the determination of serum CAT activity. Sterile water was added to a taube and 0.1 mL of enzyme solution in another tube. Buffer and substrate solutions were added to both tubes and mixed with a vortex mixer for 3 minutes. The enzyme activity was determined from the absorbance at 240 nm [21].

Activity = (2,3 / Δx) x [(log A1 / log A2)];

Activity; Calculated as in U / L.

Δx = 30 seconds

2,3 = 1 μmol optical density of H_2O_2 in 1 cm light path.

2.6. Determination of Malonaldehyde (MDA) Level

Malonaldehyde (MDA) is one of the peroxidation products formed when fatty acids and free radicals react. MDA levels were measured following the addition of thiobarbituric acid to form a colored product.

About 200 µL of a lavender serum was taken. On top of this, 800 µL of phosphate buffer and 25 µL of BHT solution and 500 µl of 30% TCA were added. The tubes were vortexed and kept in ice bath for 2 hours after the caps were closed. The tubes were brought to room temperature. Then, after the caps of the tubes were removed, they were centrifuged at 2000 rpm for 15 min. The supernatant (filtrate) obtained from the centrifuge was transferred to another tube by taking 1 ml. 75 µL of EDTA and 25 µL of TBA were added to 1 mL of the filtrates. The tubes were vortexed and held in a hot water bath for 15 min (70 ° C). Then it was brought to room temperature and read absorbance at 532 nm in UV / Vis spectrophotometer.

Concentration (µmol/L) = F x 6.41 x Abs

Where F is the dilution factor and Abs is the absorbance reading.

3. Results and discussion

The comparison results for SOD, GSH, CAT and MDA and their descriptive statistics are given in Table 1. The groups average differences were found to be statistically significant ($p < 0.05$). In accordance, GSH, SOD and CAT levels were significantly lower in patients group compared to the control group, while the MDA level was found to be quite high.

Table 1. Descriptive statistics and comparison results (results of biochemical parameters)

	Group	n	Mean ± Std. Deviation	P
SOD (U/L)	Patient	36	3.9714±0.4741	0.00112
	Control	40	7.8933±0.2058	
GSH (mmol/L)	Patient	36	0.0303±0.0065	0.00137
	Control	40	0.0685±0.0073	
CAT (U/L)	Patient	36	0.0686±0.0011	0.00165
	Control	40	0.1356±0.0167	
MDA (mmol/L)	Patient	36	11.9972±2.9092	0.00144
	Control	40	5.0653±0.0437	

A significant difference in the level of SOD has been observed between the two groups (patients and control) (Fig. 1). The mean level of SOD in control group is higher than patients group and the comparison between control and patient group level (7.893 ± 0.2058 U/L and 3.971 ± 0.4741 U/L, respectively), was found to be statistically significant ($p<0.05$).

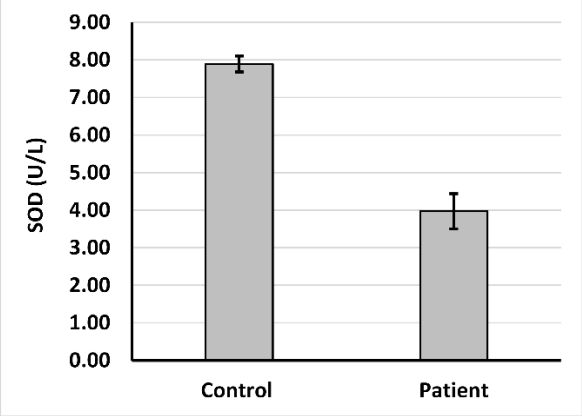

Fig. 1 Mean of serum SOD (U/L) level for patients and control group

The difference between the antioxidant level of GSH between patient group and control was found to be significant also. The RA patients have lower level of GSH compared to the control group. The relationship between control and patient group glutathione (GSH) reducing enzyme activity results (0.0685±0.00726 µmol/L and 0.03028±0.00654 µmol/L, respectively) (Table 1), were found to be statistically significant ($p<0.05$).

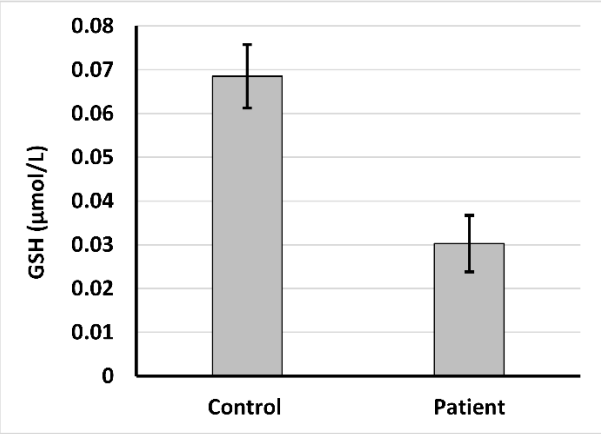

Fig. 2 Mean serum GSH (µmol/L) activity level for patients and control group

The antioxidant CAT activity difference between patients and controls is significant also and higher catalase activity was observed in control group compared to patients' group (Fig. 3).

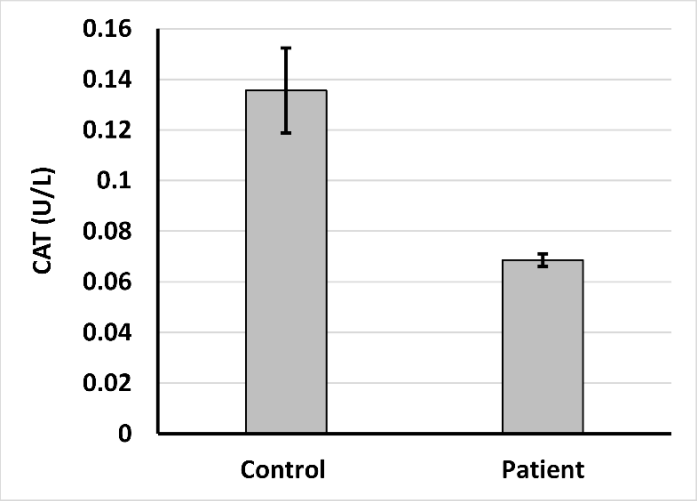

Fig. 3 Mean serum CAT (U/L) activity level for patient and control group

Figure 4 shows the level of malondialdehyde activity, which is the end product of lipid peroxidation, between control and RA patient. A significant difference was observed and clearly shows higher level of MDA activity in RA patients. When malondialdehyde (MDA) level was examined (Table 1), it was proved that there was a statistically significant relationship ($p<0.05$) between control and patient group levels (5.065±0.0437 μmol/L and 11.997±2.9505 μmol/L, respectively).

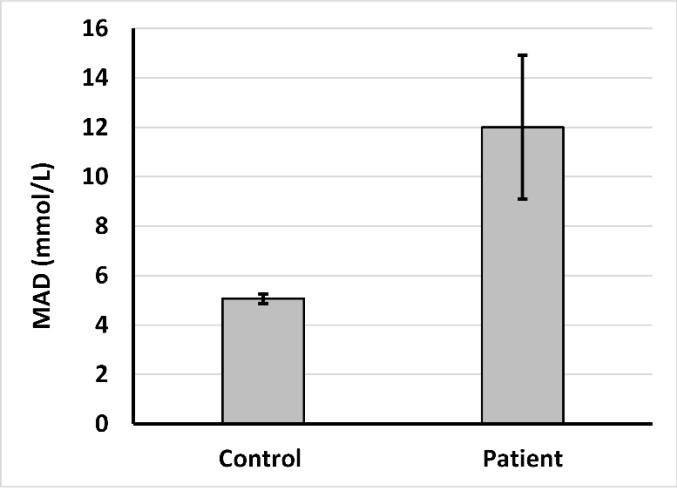

Fig. 4 Mean serum MDA (mmol/L) activity level for patient and control group

Reactive oxygen species and oxidative stress both have a role in RA pathogenesis [22]. Free radicals and other reactive species play a significant role of super oxidant leading to oxidation of biomolecules such as proteins, amino acids, lipids and DNA [23], which are eventually responsible for injury of cells and death [24]. The main attack are the polyunsaturated fatty acids in the membrane lipids leading to lipid peroxidation (LPO) which may cause disorganization of cell structure and function. additional decomposition of peroxidized lipids produces an extended variety of final-products, including malondialdehyde (MDA) [25]. Malondialdehyde (MDA) is one of a significant lipid peroxide which is high in RA patients [26]. MDA Measurement is greatly used as an indicator of LPO.

In the present study, lipid peroxidation in terms of MDA production was crucially increased in RA patients (Table 1) which might result from increased ROS during chronic inflammation. Lipid peroxides are produced at the location of tissue injury due to inflammation and it diffuses into blood and can be measured in serum or plasma [27]. Studies have reported increased levels of MDA in the plasma, serum and erythrocytes of RA patients. In our research SOD levels are greatly increased (Table 1). Superoxide anion (O_2^-) has an important role in the pathogenesis of many diseases [28]. It is neutralized by SOD to hydrogen peroxide (H_2O_2). H_2O_2 is further ended by activity of both glutathione peroxidase and catalase. conversion of O_2^- to H_2O_2 prevents the creation of harmful compound such as peroxynitrile ($ONOO^-$) and hydroxyl radical (OH^-) [29]. The patients revealed significantly higher activity of SOD (Table 1).

Many studies have reported elevated MDA level in the serum, plasma and synovial fluid of RA patients [30]. MDA plays an important role in pathogenesis of RA. There is developing awareness that reactive oxygen species and free radicals may play a crucial part in rheumatoid arthritis' cellular injury and tissue damage mediation. It was reported that malondialdehyde-acetaldehyde (MAA) adduct production is increased in RA [31]. They

seem to result in robust antibody reactions which are strongly linked with anti-citrullinated protein antigens (ACPAs) suggesting that MAA production may be a cofactor that causes tolerance loss, which leads to the autoimmune responses characteristic of RA. But perhaps elevated activity of SOD may be attributed to increased production of O_2- by hyperactive cells leading to enhancing SOD activity [32]. Another possibility may be extreme free radical generation through the xanthine-xanthine oxidase system is the basic factors in RA, rather than a weakened antioxidant system [33]. Else higher levels of SOD may be a change to stop over production of free radical. Post treatment the antioxidants are raised which lead to minimize plasma MDA and increased total capacity of antioxidant (TAC) [34]. Extra generation of extracellular SOD causes dismutation of superoxide resulting in H_2O_2 accumulation as a consequence. Analysis of H_2O_2 in various settings is being carried out and authors conclude, more SOD does not indicate more H_2O_2 [35]. The generation of H_2O_2 because of dismutation of superoxide is restricted by the amount of superoxide, not by the rate it is converted to H_2O_2. superoxide accumulation leads to the oxidation of NO yielding peroxynitrite. There more H_2O_2 is improbably to be toxic as this would amount to replacing a very mild cytokine (H_2O_2) for a powerful (peroxynitrite) [36]. Catalase activity was not discovered in serum of RA patients. Our study presented lower activity of catalase in serum of RA patients. Lower catalase level may be responsible for high inflammation in RA [37]. In the current study, we have noticed an important decrease in the catalase activity in rheumatoid arthritis patients compared to controls. Catalase is the enzyme, which protects the cells from the buildup of hydrogen peroxide by dismutating it to generate water and oxygen or by consuming it as an oxidant in which it serves as a peroxidase [38]. There have been similar reports of reduced catalase activity in rheumatoid arthritis by Kerimova et al [39]. However, others have discovered an elevation in activity of plasma catalase in patients with rheumatoid arthritis when it comes to be compared to controls [40]. Jalili et. al. [41] indicated that antioxidants may significantly improve activity of disease but do not

influence the number of swollen and painful joints. Thus, antioxidants may be useful in managing of clinical results and oxidative stress in RA patients.

4. Conclusion

Oxidative stress happened by ROS increases the risk of several disorders, including inflammatory diseases, cardiovascular disease, diabetes, cancer, Alzheimer's disease, cataracts, autism and aging. Antioxidants can directly interact with the reactive radicals to destroy them by receiving or giving an electron(s) to remove the unpaired radical conditions, or they may indirectly decrease the generation of free radicals by restricting the activities or limiting of free radical generating enzymes or by intensifying both expressions activities and of other antioxidant enzymes.

In conclusion oxidative stress management may be regarded as RA treatment option along with DMARD. Combining catalase and/or GPX with antioxidant supplements may increase protection.

References

1 Deligiannidou GE, Gougoula V, Bezirtzoglou E, Kontogiorgis C, Constantinides TK (2021) The Role of Natural Products in Rheumatoid Arthritis: Current Knowledge of Basic In Vitro and In Vivo Research. **Antioxidants,** 10(4), 599.

2 Andersson ML, Svensson B, Forslind K (2021) Distribution of erosions in hands and feet at the time for the diagnosis of RA and during 8-year follow-up**. Clin Rheumatol** 40, 1799–1810.

3 Grellmann C, Dombrowsky W, Fabricius V (2021) Epidemiology and Treatment of Patients with Rheumatoid Arthritis, Psoriatic Arthritis and Psoriasis in Germany: A Real-World Evidence Study**. Adv Ther** 38, 366–385.

4 Quiñonez-Flores C. M, González-Chávez S. A. (2016) Oxidative stress relevance in Nájera, and C. Pacheco-Tena, the pathogenesis of the rheumatoid arthritis: a Systematic, Article ID review, **BioMed Research International**, 6097417.

5 DeMizio DJ, Geraldino-Pardilla LB (2020) Autoimmunity and Inflammation Link to Cardiovascular Disease Risk in Rheumatoid Arthritis. **Rheumatol Ther** 7, 19–33.

6 Kemeny-Beke A, Szodoray P. (2020) Ocular manifestations of rheumatic diseases. **Int Ophthalmol** 40, 503–510.

7 Scott D. L., Wolfe F., Huizinga T. W. (2010) "Rheumatoid arthritis," **Lancet**, vol. 376, no. 9746, pp. 1094–1108.

8 Guerra JD, De Santiago AB, Reed S (2022) Cardiology co-management of rheumatoid arthritis patients with coronary artery disease as an intervention reduces hospitalization rates and adverse event occurrence. **Clin Rheumatol**. https://doi.org/10.1007/s10067-022-06335-4

9 Wang L, Tan W, Wang F (2018) Artery compliance in patients with rheumatoid arthritis: results from a case-control study. **Clin Rheumatol** 37, 169–177.

10 Seabra Rato M, Bernardo A, Oliveira Pinheiro F (2022) The skin as the first sign of a rare complication of rheumatoid arthritis. ***Clin Rheumatol***. https://doi.org/10.1007/s10067-022-06303-y

11 Widdifield J, Paterson JM, Huang A, Bernatsky S. (2018). Causes of death in rheumatoid arthritis: How do they compare to the general population? ***Arthritis Care & Research.***

12 Leyane TS, Jere SW, Houreld NN (2022) Oxidative Stress in Ageing and Chronic Degenerative Pathologies: Molecular Mechanisms Involved in Counteracting Oxidative Stress and Chronic Inflammation. **Int. J. Mol. Sci.**, 23, 7273. https:// doi.org/10.3390/ijms23137273

13 Ikonomidis I., Tzortzis S., Andreadou I., Paraskevaidis I., Katseli C., Katsimbri P., Lekakis J. (2014) Increased Benefit of Interleukin-1 Inhibition on Vascular Function, Myocardial Deformation, and Twisting in Patients With Coronary Artery Disease and Coexisting Rheumatoid Arthritis. Circulation: **Cardiovascular Imaging**, 7(4), 619–628.

14 Jalili M, Kolahi S, Aref-Hosseini SR, Mamegani ME, Hekmatdoost A, (2014) Beneficial role of antioxidants on clinical outcomes And, erythrocyte antioxidant parameters in rheumatoid arthritis patients. **Int J, and Prev Med** 5: 835-840.,

15 Roy A, Das S, Chatterjee I, Roy S, Chakraborty R (2022) Anti-inflammatory Effects of Different Dietary Antioxidants. In: Ekiert, H.M., Ramawat, K.G., Arora, **J. (eds) Plant Antioxidants and Health**. https://doi.org/10.1007/978-3-030-78160-6_20

16 Tsikas D, Mikuteit M (2022) N-Acetyl-L-cysteine in human rheumatoid arthritis and its effects on nitric oxide (NO) and malondialdehyde (MDA): analytical and clinical considerations. **Amino Acids** 54, 1251–1260. https://doi.org/10.1007/s00726-022-03185-x

17 Mobasheri A, Biesalski HK, Shakibaei M, Henrotin Y (2014) Antioxidants and Osteoarthritis. In: Laher, I. (eds) **Systems Biology of Free Radicals and Antioxidants**. Springer, Berlin, Heidelberg.

18 Wang Z, Wang W, Xiang T (2022) Serum Uric Acid as a Diagnostic Biomarker for Rheumatoid Arthritis–Associated Interstitial Lung Disease. **Inflammation** 45, 1800–1814. https://doi.org/10.1007/s10753-022-01661-w

19 Lee J.E, Kim I.J, Cho M.S, Lee J. (2017) A Case of Rheumatoid Vasculitis Involving Hepatic Artery, and in Early Rheumatoid Arthritis. **J Korean Med Sci** 32:1207-1210.

20 Ahmed A.S, Eryilmaz R., Demir H., Aykan S., Demir C. (2018). Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. **The Aging Male**, 1–9.

21 Mateen S, Moin S, Khan AQ, Zafar A, Fatima N (2016) Increased Reactive Oxygen Species Formation and Oxidative Stress in Rheumatoid Arthritis. **PLoS ONE** 11(4): e0152925.

22 Browne SE, Beal MF (2006) Oxidative damage in huntington's disease pathogenesis. **Antioxid Redox Signal** 8:2061-2073.

23 Nagy P, Kettle AJ, Winterbourn CC (2009) Superoxide-mediated formation of tyrosine hydroperoxides and methionine sulfoxide in peptides through radical addition and intramolecular oxygen transfer. **J Biol Chem.** 284(22): p. 14723-33.

24 Van der Linden M.P, Nevel K.R, Huizinga T.W, Van der Helmvan M.A.H. (2011) Classification of rheumatoid, arthritis: comparison of the 1987 American College of Rheumatology criteria and the 2010 American, College of Rheumatology/European League Against Rheumatism criteria. **Arthritis Rheum.** 63:, and 37–42.

25 Batooei M., Tahamoli-Roudsari A., Basiri Z., Yasrebifar F., Shahdoust M., Eshraghi A., Ataei S. (2018) Evaluating the Effect of Oral N-acetylcysteine as an Adjuvant Treatment on Clinical Outcomes of Patients with Rheumatoid Arthritis: A Randomized, Double Blind Clinical Trial. **Reviews on Recent Clinical Trials**, 13(2), 132–138.

26 Smallwood M.J, Nissim A, Knight A.R, Whiteman M, Haigh R, Winyard P.G. (2018) Oxidative stress in autoimmune rheumatic diseases. **Free Radic Biol Med**. 125 :3–14.

27 Mezes M, Bartosiewicz G. (1983) Investigations on vitamin E and, lipid peroxide status in rheumatic diseases. **Clin Rheumatol**, 2(3): 259-63.

28 Tanabe S, O'Brien J, Tollefsen KE et. al. (2022) Reactive Oxygen Species in the Adverse Outcome Pathway Framework: Toward Creation of Harmonized Consensus Key Events. **Front.Toxicol**. 4:887135. doi: 10.3389/ftox.2022.887135

29 Mishra R, Singh A, Chandra V, Negi MP, Tripathy BC, et al. (2012) A, comparative analysis of serological parameters and oxidative stress In, and osteoarthritis and rheumatoid arthritis. **Rheumatol Int** 32: 2377-2382

30 Das DC, Jahan I, Uddin MG et al. (2021)Serum CRP, MDA, Vitamin C, and Trace Elements in Bangladeshi Patients with Rheumatoid Arthritis. **Biol Trace Elem Res** 199, 76–84.

31 Lomzenski HE, Thiele GM, Duryee MJ et al. (2022) Serum anti-malondialdehyde-acetaldehyde IgA antibody concentration improves prediction of coronary atherosclerosis beyond traditional risk factors in patients with rheumatoid arthritis. **Sci Rep** 12, 10547. https://doi.org/10.1038/s41598-022-14954-9

32 Chalan P., Bijzet J., van den Berg A., (2016) Analysis of serum, immune markers in seropositive and seronegative Rheumatoid, arthritis and in high-risk seropositive arthralgia Patients. **Scientific Reports**, vol. 6, no. 1

33 Pallinti V, Ganesan N, Anbazhagan M, Rajasekhar G (2009) Serum, biochemical markers in rheumatoid arthritis. **Indian J Biochem Biophys**, 46: 342-344.

34 Bordy R., Totoson P., Prati C., Marie C., Wendling D., (2018) Microvascular endothelial dysfunction in C. Demougeot, vol. 14 rheumatoid arthritis, **Nature Reviews Rheumatology**, 7, 404–420

35 Cintia PG, Leonardo M, Israel ORW et al. (2016) Superoxide Dismutase Activity, Hydrogen Peroxide Steady-State Concentration, and Bactericidal and Phagocytic Activities Against Moraxella bovis, in Neutrophils Isolated from Copper-Deficient Bovines. **Biol Trace Elem Res** 171, 94–100.

36 Türk S.M., Cansu D.Ü., Teke H.Ü., Kaşifoğlu T., Meltem Akay O., Bilgin M., Korkmaz C. (2018). Can we predict thrombotic tendency in rheumatoid arthritis? A thromboelastographic analysis (with ROTEM). **Clinical Rheumatology**, 37(9), 2341–2349.

37 Mateen S., Moin S., Khan A.Q., Zafar A., Fatima N., (2016) Increased reactive oxygen species formation and Oxidative, no. 4 stress in rheumatoid arthritis. **PLoS One**, vol. 11, p. e0152925

38 Raducan, A., Cantemir, A.R., Puiu, M. et al. (2012) Kinetics of hydrogen peroxide decomposition by catalase: hydroxylic solvent effects. **Bioprocess Biosyst Eng** 35, 1523–1530.

39 Crowley T., O'Neil J.D., Adams H., Thomas A.M., Filer A., Buckley C.D., Clark A.R. (2017). Priming in response to pro-inflammatory cytokines is a feature of adult synovial but not dermal fibroblasts. **Arthritis Research & Therapy**, 19(1).

40 Chen Z., Andreev D., Oeser K., Krljanac B., Hueber A., Kleyer A., Bozec A. (2016). Th2 and eosinophil responses suppress inflammatory arthritis. **Nature Communications**, 7, 11596.

41 Zaghloul N, Patel H, Codipilly C, Marambaud P, Dewey S, (2014) Overexpression of Extracellular Superoxide Dismutase Protects Against, and Brain Injury Induced by Chronic Hypoxia. **PLoS One** 9: E108168.