RESEARCH ARTICLE

Comparison of oxidative stress, lipid peroxidation and inflammatory markers between rheumatoid arthritis and ankylosing spondylitis patients

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Abstract

Objective: To measure the levels of superoxide dismutase and malondialdehyde along with erythrocyte sedimentation rate and C-reactive protein in patients of rheumatoid arthritis and ankylosing spondylitis.

Method: The comparative, cross-sectional study was conducted from February 2 to December 30, 2022, at the Centre for Research in Experimental and Applied Medicine laboratory of the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, Pakistan, in collaboration with the Department of Rheumatology, Pak Emirates Military Hospital, Rawalpindi. The sample comprised healthy controls in group 1, patients of rheumatoid arthritis in group 2 and patients of ankylosing spondylitis in group 3. Blood samples were assessed for levels of superoxide dismutase, malondialdehyde, erythrocyte sedimentation rate and C-reactive protein. Data was analysed using SPSS 25.

Results: Of the 180 subjects, 60(33.3%) were in group 1; 32(53.3%) females and 28(46.7%) males with mean age 34.9±6.4 years. There were 60(33.3%) patients in group 2; 35(58.3%) females and 25(41.7%) males with mean age 46.0±11.1 years. There were 60(33.3%) patients in group 3, and all 60(100%) were males with mean age 35.9±6.9 years. Superoxide dismutase level was significantly low and malondialdehyde level was significantly high in groups 2 and 3 compared to group 1 ($p<0.05$). Erythrocyte sedimentation rate was the highest in group 2, followed by group 3 ($p<0.05$). C-reactive protein levels were the highest in group 2 and the lowest in group 3 ($p<0.05$). A significantly negative correlation ($p<0.001$) was found between superoxide dismutase and malondialdehyde.

Conclusion: Oxidative stress played a pivotal role in chronic inflammatory rheumatic diseases, like rheumatoid arthritis and ankylosing spondylitis.

Keywords: Rheumatoid arthritis, Ankylosing spondylitis, Superoxide dismutase, Malondialdehyde. (JPMA 74: 886; 2024)

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Introduction

Rheumatoid arthritis (RA) and spondyloarthropathies hold a pivotal position in the category of inflammatory arthritis in rheumatic diseases. Among the spondyloarthropathies, ankylosing spondylitis (AS) is considered the prototype.[1] These diseases have a chronic autoimmune nature, and widespread inflammation is their pathophysiological basis. Synovitis is considered the characteristic feature of RA which generally involves the diarthrodial joints. On the contrary, AS primarily affects the axial skeleton which may be accompanied by articular manifestations of the peripheral skeleton.[2] Manifestation of these rheumatic diseases is an interplay of both genetic and epigenetic factors. The presence of human leukocyte antigen DR4 (HLA-DR4) enhances the susceptibility of individuals to develop RA,[3] whereas HLA-B27 characterises patients of spondyloarthropathies, including AS.[4] Sex hormones, infections of bacterial or viral origin and smoking are some of the epigenetic factors positively contributing towards the expression of these diseases. The exact biochemical basis of RA and AS has still not been ascertained, but the role of oxidative stress (OS) has been widely proposed to be contributing towards the inflammatory process and subsequent deterioration of synovial structures.[5] OS has generally been defined as an imbalance between the level of reactive oxygen species (ROS) and the enzymatic and non-enzymatic antioxidants in the body. It may be a consequence of raised oxidants production, deficient antioxidants levels or a combined effect of the two.[6] OS has a great impact on human machinery, and is said to be the biochemical basis of many chronic inflammatory disorders and even malignancies. Many factors act as the contributory factors to OS. Nutrition and diet, stress and trauma, smoking and alcohol are known to augment the OS state. Among the various enzymatic antioxidants, superoxide dismutase (SOD) has endogenous production. Superoxide and other free radicals contribute to the oxidation of biomolecules, like proteins, amino acids, lipids, and deoxyribonucleic acid (DNA), which, in turn, causes cell damage and death. The polyunsaturated fatty acids in the membrane lipids are the main targets of ROS attack, resulting in lipid peroxidation (LPO), which can disrupt cell

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structure and function. Malondialdehyde (MDA) is one of the final products produced by the further breakdown of peroxidised lipids.[7]

The current study was planned to measure the levels of SOD and MDA along with erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in patients of RA and AS.

Material/Subjects/Patients and methods

The comparative, cross-sectional study was conducted from February 2 to December 30, 2022, at the Centre for Research in Experimental and Applied Medicine (CREAM) laboratory of the Department of Biochemistry and Molecular Biology, Army Medical College (AMC), Rawalpindi, Pakistan, in collaboration with the Department of Rheumatology, Pak Emirates Military Hospital (PEMH), Rawalpindi. After approval from the AMC ethics review board, the sample size was estimated using the World Health Organisation (WHO) calculator with prevalence 1.5%, confidence interval (CI) 95% and error 5%.[8,9] The sample was raised using non-probability purposive sampling technique. Those included were individuals of either gender aged between 25-70 years. The sample was divided into healthy controls group 1, RA patients group 2 and AS patients group 3. Those outside the age bracket or suffering from hypertension and diabetes or cardiac, renal or hepatic diseases or having any malignancy were excluded.

The diagnoses of RA and AS were made as per the standard criteria.[10,11]

After taking informed consent from the participants, 5ml blood was withdrawn under aseptic measures; 2 mL for ESR and CRP testing at the Combined Military Hospital (CMH) laboratory, and 3 ml in serum separator vacutainers for the estimation of SOD and MDA levels. The vacutainers were safely transported to the AMC Multidisciplinary Lab-1 in an ice bag. Serum SOD levels were assessed using human SOD-1 enzyme-linked immunosorbent assay (ELISA) kit (Catalogue no E-EL-H1113, Elabscience-USA) and MDA was assessed using another ELISA kit (Catalogue No E-EL-0060, Elabscience- USA).

Data was analysed using SPSS 25. Continuous variables were expressed as mean ± standard deviation, whereas frequencies and percentages were used for categorical data. Comparison of continuous data among the 3 groups was done using Kruskal Wallis test, which was followed by post-hoc Dunn test for pairwise analysis. Data was subjected to Pearson's correlation test for finding the correlation among the markers. P<0.05 was regarded as statistically significant.

Results

Of the 180 subjects, 60(33.3%) were in group 1; 32(53.3%) females and 28(46.7%) males with mean age 34.9±6.4 years. There were 60(33.3%) patients in group 2; 35(58.3%) females and 25(41.7%) males with mean age 46.0±11.1 years. There were 60(33.3%) patients in group 3, and all 60(100%) were males with mean age 35.9±6.9 years.

The values of SOD and MDA were noted for all the groups (Figure). SOD levels were decreased in groups 2 and 3 compared to group 1 (p<0.001). Levels of MDA were significantly raised in groups 2 and 3 compared to group 1 (p<0.001). The median of ESR was significantly raised in group 2 patients 24(IQR-28), followed by group 3 14(IQR-22) and 8(IQR-2) in group 1. CRP levels were the highest in group 3 10.8(12.4), followed by group 1 9 (3.0) and group 2 4.8(6.9).

Significant difference (p<0.001) for SOD and MDA was observed between group 1 compared to groups 2 and 3, but the difference was not significant between group 2 and 3 (Table 1).

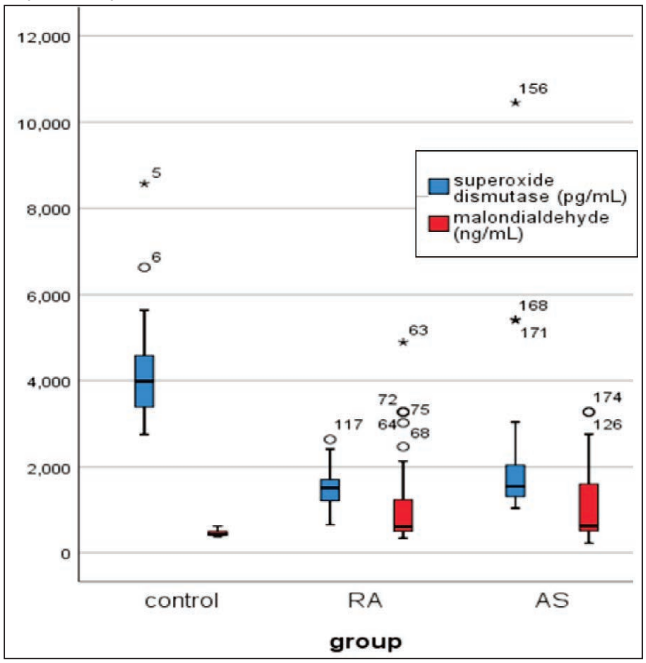

Figure: Comparison of SOD-1 and MDA values among the groups.
SOD: Superoxide dismutase, MDA: Malondialdehyde, RA: Rheumatoid arthritis, AS: Ankylosing spondylitis.

Table-1: Intergroup comparison of the studied parameters.

Parameters	Group I v/s		Group II v/s
	Group II	Group III	Group III
SOD-1	< 0.001	< 0.001	0.145
MDA	< 0.001	< 0.001	0.795
ESR	< 0.001	< 0.001	0.035
CRP	0.770	< 0.001	0.001

SOD: Superoxide dismutase, MDA: Malondialdehyde, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein.

Table-2: Correlation of SOD-1 with MDA, ESR and CRP.

Study variable	Parameters correlated	*r*- value	*p*-value
Serum SOD1 (pg/mL)	MDA (ng/mL)	-0.314	< 0.001
	ESR	-0.363	< 0.001
	CRP	-0.085	0.258

SOD: Superoxide dismutase, MDA: Malondialdehyde, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein.

A significant negative correlation was found between SOD and MDA, and between SOD and ESR, while no appreciable correlation was found between SOD and CRP (Table 2).

Discussion

The current study showed the trend of decline in the total antioxidant capacity (TAC) in rheumatic diseases and a simultaneous upsurge in the levels of oxidative markers, like MDA. A study reported this trend in Iraqi RA patients.[12] In a similar study on AS patients, TAC decreased significantly compared to healthy controls, whereas the AS patients exhibited no variation in the total oxidant status (TOS).[13]

The current study revealed a decline in the levels SOD in RA and AS compared to the controls, whereas MDA levels was significantly raised in both groups compared to the controls. The outcome was consistent with earlier findings among newly-diagnosed RA patients who had not received any treatment. The levels of enzymatic antioxidants SOD and glutathione peroxidase (GPx) were significantly lowered. Xanthine oxidase (XO) activity on the other hand was significantly higher in the patients. MDA along with the inflammatory marker CRP was also significantly raised, hence, validating the OS state.[14]

RA and AS presentation is a picture of prolonged underlying inflammation. OS over a long period of time, particularly in the synovium of RA patients, has been held responsible for bringing about an increase in intra-articular pressure, subsequently leading to augmented production of ROS as a by-product of the electron transport chain and oxidative phosphorylation occurring in the mitochondria. This phenomenon results in recurrent episodes of hypoxia, followed by re-oxygenation. A high production and replication of cells as a response to the ongoing inflammation serves as the most viable explanation for the hypoxic state in RA patients.[15]

Many inflammatory mediators, such as cytokines and interleukins (ILs) have a central role in bringing about the visible changes in these diseases. In a study on RA patients, the levels of IL1β, IL6 and tumour necrosis factor-alpha (TNF-α) were found to be higher in addition to the antioxidant SOD. Catalase (CAT) and GPx were also raised. MDA levels indicated significant LPO and was also elevated.[16]

In AS patients, the same trend was shown for SOD, nitric oxide (NO), CAT and TOS along with the TNF and IL1β.[17] Another research on AS patients revealed an increase in SOD and glutathione (GSH) levels in patients with metabolic syndrome.[18] The finding of these studies regarding SOD levels is not in agreement with the current results, but fluctuations in the levels of SOD have also been observed in rheumatic diseases, based upon the differences in the phase of the disease. Generally, the rise in SOD levels is attributed to the initial time period of the active phase. The phenomenon can be explained as a result of excessive superoxide radical being produced by the hyperactive cells, and the SOD being the first-line antioxidant defence, that try to counteract the increased free radical's production. This contradiction in the levels of antioxidants in rheumatic diseases has been cited in other studies as well, like a study in Pakistani population that showed rise in SOD levels in RA patients along with rise in MDA levels.[18]

Among the category of enzymatic antioxidants, SOD, GPx and CAT have been the subject of extensive research for evaluating OS. The result of Mahdi et al. was in congruence with the current results where the levels of SOD declined and those of MDA were significantly raised in RA group compared to the controls. The current finding that SOD and MDA had a significant negative correlation was also consistent with the findings of Mahdi et al.[20]

Anwar et al. conducted a study comparing OS in RA patients and healthy controls. In addition to the enzymatic antioxidants SOD, CAT and GPx, vitamin A, C and E were also included for evaluation along with NO and MDA. All the antioxidants had decreased levels compared to the controls. NO was found to be elevated in RA patients, representing its role in inflammation. The outcome of MDA followed the trend of rise, indicating pronounced LPO.[21]

Newer avenues are being explored currently that are pertinent to the domain of OS and subsequent utilisation of the knowledge in the prompt diagnosis and effective management of diseases. Therapies that aim at the mitigation of oxidants or enhancement of antioxidant levels may prove to be fruitful in the management of such diseases.[21] Among such options, supplementation with extract of pomegranate, symbiotic and sesamin supplementation, the use of coenzyme Q10 and N-acetyl cysteine, as well as laser acupuncture have shown improvement in the levels of ESR and disease activity score (DAS-28) in RA patients.[22]

In a study conducted on mice suffering from AS, injections of human proteoglycan extract IQW (Ile-Gln-Trp) led to amelioration in the levels SOD and decline in MDA, thus

proposing the possible beneficial effects of IQW in the treatment and management of AS..[23] Synthetic antioxidant preparations as a treatment modality is a topic of research in various malignancies, the possible beneficial role of these preparations in rheumatic diseases might be explored by medical researchers.

The current study has limitations as owing to financial constraints, only two OS markers, SOD and MDA, were measured and compared. Also, the sample was small and raised from a single centre. Multicentre studies with larger sample sizes are recommended for more reliable results.

Conclusion

OS and inflammation were the two processes that together played a major role in the disease pathogenesis of rheumatic diseases, such as RA and AS.

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Author Contribution:

SK: Concept, design, data analysis, sampling, wet lab work.
JY: Critical review of intellectual content.
AR: Final approval.
AM: Assistance and intellectual assistance in wet lab.
UH: Concept, design, data analysis and interpretation.
AJ: Revision and final approval, assistance in sampling and wet lab procedure, statistical analysis.