# Comparison of Oxidative Stress, Lipid Peroxidation and Inflammatory Markers Between Rheumatoid Arthritis and Ankylosing Spondylitis Patients

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**Abstract**

**Background:** Rheumatoid arthritis (RA) and spondyloarthropathies are the most common types of inflammatory arthritis among rheumatic patients. Ankylosing spondylitis (AS) is regarded as the most common example of spondyloarthropathies. Both RA and AS are chronic autoimmune diseases that cause extensive inflammation in the joints. Oxidative stress (OS) is commonly defined as an imbalance between reactive oxygen species (ROS) and the body's enzymatic and non-enzymatic antioxidants. Superoxide dismutase (SOD) is an enzyme antioxidant that is produced naturally in the body. **Objective:** To assess Superoxide dismutase and MDA levels, as well as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in patients with rheumatoid arthritis and ankylosing spondylitis. **Study design:** A comparative study. **Place and Duration:** This study was conducted in Liaquat University Hospital, Hyderabad, Sindh, Pakistan from May 2021 to May 2022. **Methodology:** Non-probability purposive sampling was used to choose participants, who ranged in age from 25 to 70 years. The participants were placed into three groups: healthy controls (Group 1), rheumatoid arthritis patients (Group 2), and ankylosing spondylitis patients (Group 3). After receiving permission from the participants, 5 milliliters of blood were drawn in a clean manner, 2 milliliters of serum were tested for ESR and CRP, while 3 millimeters were separated into specific tubes to measure Superoxide dismutase and MDA levels. **Results:** There were a total of 210 participants of this study out of which 132 (62.8%) were males and 78(37.2%) were females. The participants were placed into three groups: healthy controls (Group 1), rheumatoid arthritis patients (Group 2), and ankylosing spondylitis patients (Group 3). The overall average age calculated was 38.9 years. There were lower levels of SOD in both rheumatoid arthritis and ankylosing spondylitis patients than in controls, although MDA levels were significantly higher in both groups. **Conclusion:** Oxidative stress and inflammation are significant in the development of rheumatoid arthritis and ankylosing spondylitis.

Keywords: Rheumatoid Arthritis, Ankylosing Spondylitis, Superoxide Dismutase, Malondialdehyde, CRP, ESR.

## Introduction

Rheumatoid arthritis (RA) and spondyloarthropathies are the most common types of inflammatory arthritis among rheumatic patients [1]. Ankylosing spondylitis (AS) is regarded as the most common example of spondyloarthropathies [2]. Both RA and AS are chronic autoimmune diseases that cause extensive inflammation in the joints [3]. The defining aspect of RA is synovitis, which affects the diarthrodial joints [4]. In contrast, AS typically affects the axial skeleton, however it can also affect peripheral joints.

The manifestation of various rheumatic disorders is caused by a mix of hereditary and epigenetic factors. Individuals with human leukocyte antigen DR4 (HLA-DR4) are more likely to develop RA, whereas HLA-B27 is a marker for patients with spondyloarthropathies, such as ankylosing spondylitis [5]. Sex hormones, bacterial or viral infections, and smoking all have a substantial impact on disease expression [6]. Although the specific biochemical pathways behind RA and AS are unknown, oxidative stress is commonly thought to play an important role in promoting inflammation and synovial tissue deterioration [7].

Oxidative stress is commonly defined as an imbalance between reactive oxygen species (ROS) and the

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body's enzymatic and non-enzymatic antioxidants [8]. This imbalance can be caused by an increase in oxidant production, insufficient antioxidant levels, or a combination of the two [9]. OS has a substantial impact on biological functioning and is thought to serve as a biochemical basis for many chronic inflammatory illnesses, including cancer [10].

Many factors contribute to oxidative stress, including stress, food, smoking, trauma, and alcohol use [11]. Superoxide dismutase (SOD) is an enzyme antioxidant that is produced naturally in the body [12]. Superoxide and other free radicals oxidize biomolecules such as amino acids, proteins, lipids, and DNA, causing cell damage and death. ROS primarily targets polyunsaturated fatty acids in membrane lipids, causing lipid peroxidation (LPO) and disrupting cell structure and function. Malondialdehyde (MDA), a byproduct of lipid breakdown, is a marker for this process [13]. The purpose of this study was to assess SOD and MDA levels, as well as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in patients with RA and AS.

## Methodology

The Ethical Review Committee approved this research. The sample size was calculated using the World Health Organization's (WHO) calculator, using a 1.5% prevalence, a 95% confidence interval (CI), and a 5% margin of error. Non-probability purposive sampling was used to choose participants, who ranged in age from 25 to 70 years. The participants were placed into three groups: healthy controls (Group 1), rheumatoid arthritis patients (Group 2), and ankylosing spondylitis patients (Group 3).

**Exclusion criteria:** Individuals beyond the specified age range were not included as well as those suffering from hepatic disorders, hypertension, cardiac, diabetes, renal, or any kind of cancer.

After receiving permission from the participants, 5 milliliters of blood were drawn in a clean manner, 2 milliliters of serum were tested for ESR and CRP, while 3 millimeters were separated into specific tubes to measure SOD and MDA levels. These tubes were subsequently transported to the laboratory in a cooler bag containing ice. Human SOD-1 levels were measured using a particular kit known as an enzyme-linked immunosorbent assay (ELISA), while MDA levels were measured using another ELISA kit.

The data was analyzed with SPSS version 25 software. Continuous variables were provided as mean ± SD, whereas categorical data were expressed as frequencies and percentages. To compare continuous data between the three groups, the Kruskal-Wallis test was employed, followed by the post-hoc Dunn test for pairwise comparisons. Pearson's correlation test was used to determine the correlations between the markers. A p-value of <0.05 was judged statistically significant.

## Results

There were a total of 210 participants of this study out of which 132 (62.8%) were males and 78 (37.2%) were females. The participants were placed into three groups: healthy controls (Group 1), rheumatoid arthritis patients (Group 2), and ankylosing spondylitis patients (Group 3). The overall average age calculated was 38.9 years. Table number 1 shows the distribution of the participants according to their gender and group.

**Table No. 1: Distribution of the Participants According to Their Gender and Group. (N=210).**

| Participants | n | % |
|---|---|---|
| • Group 1 | • | • |
| • Male | • 37 | • 52.8 |
| • Female | • 33 | • 47.2 |
| • Group 2 | • | • |
| • Male | • 41 | • 58.5 |
| • Female | • 29 | • 41.5 |
| • Group 3 | • | • |
| • Male | • 70 | • 100 |
| • Female | • 0 | • 0 |

Table number 2 shows intergroup comparison of the studied parameters.

**Table No. 2: Intergroup Comparison of the Studied Parameters.**

| Parameters | Group 1 v/s | | Group 2 v/s Group 3 |
|---|---|---|---|
| | Group 2 | Group 3 | |
| CRP | 0.77 | <0.001 | 0.001 |
| ESR | <0.001 | <0.001 | 0.035 |
| MDA | <0.001 | <0.001 | 0.795 |
| SOD-1 | <0.001 | <0.001 | 0.001 |

Table number 3 shows the link of SOD-1 with ESR, MDA, and CRP.

**Table No. 3: LINK oF Sod-1 WITH Esr, Mda, AND Crp.**

| Variable | Parameters correlated | r-value | p-value |
|---|---|---|---|
| Serum SOD-1 (pg./mL) | ESR | -0.363 | <0.001 |
| | MDA | -0.314 | <0.001 |
| | CRP | -0.085 | 0.258 |

## Discussion

The study discovered a decrease in total antioxidant capacity (TAC) and an increase in oxidative indicators like MDA in rheumatic illnesses [14]. This pattern was also observed in a study of Iraqi individuals with rheumatoid arthritis [15]. Similarly, in a study of ankylosing spondylitis patients, TAC was much lower than in healthy controls, while total oxidant status (TOS) remained the same [16].

The current study discovered lower levels of SOD in both rheumatoid arthritis and ankylosing spondylitis patients than in controls, although MDA levels were significantly higher in both groups. These findings are consistent with prior research in newly diagnosed RA patients who had not received treatment, in which levels of enzyme antioxidants such as SOD and glutathione peroxidase (GPx) were significantly lowered [17]. Furthermore, xanthine oxidase (XO) activity was dramatically increased in these patients. Elevated MDA levels, together with the inflammatory marker CRP, demonstrated the existence of oxidative stress.

The appearance of rheumatoid arthritis and ankylosing spondylitis reflects chronic inflammation. Over time, oxidative stress, notably in the synovium of RA patients, has been linked to increased intra-articular pressure, resulting in increased formation of reactive oxygen species (ROS) as a consequence of mitochondrial electron transport chain and oxidative phosphorylation [18]. This process causes repeated cycles of hypoxia and reoxygenation. The primary causes of hypoxia in RA patients include increased cell formation and replication in response to continuous inflammation.

Numerous inflammatory mediators, such as cytokines and interleukins (ILs), contribute significantly to the observed alterations in these circumstances. A study on RA patients found raised levels of IL1β, IL6, and TNF-α, as well as increased levels of the antioxidant SOD. CAT and glutathione peroxidase (GPx) levels were also enhanced [19]. Notably, levels of Malondialdehyde showed considerable lipid peroxidation and were raised.

SOD, GPx, and CAT are well-studied enzymatic antioxidants used to measure oxidative stress. Mahdi et al. found lower levels of SOD and higher amounts of MDA in the RA group compared to controls, which are consistent with our current findings. Furthermore, our discovery of a substantial negative connection between SOD and MDA corresponds to the findings of Mahdi et al [20].

Due to financial constraints, only two oxidative stress markers, SOD and MDA, were evaluated and compared in the present investigation. Furthermore, the sample size was modest and drawn from a single center. For more accurate results, multicenter research with bigger sample sizes is recommended.

## Conclusion

Oxidative stress and inflammation are significant in the development of rheumatoid arthritis and ankylosing spondylitis

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This study was conducted without receiving financial support from any external source.

### Conflict in the interest

The authors had no conflict related to the interest in the execution of this study.

### Permission

Prior to initiating the study, approval from the ethical committee was obtained to ensure adherence to ethical standards and guidelines.

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