

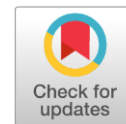
ORIGINAL RESEARCH ARTICLE

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Lipid Peroxidation and Detection of Pro-Inflammatory Variables in Rheumatoid Arthritis- A Comprehensive Analysis of Malondialdehyde and Isoprostanes in Synovial Fluids and SERA

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease marked by systemic inflammation and joint destruction produced mostly by oxidative stress and lipid peroxidation. Analyzing markers for these processes is critical to understanding the etiology of RA.

Objectives: The goal of this study is to assess the importance of the lipid peroxidation indicators malondialdehyde (MDA) and isoprostanes in RA synovial fluids and sera in indicating oxidative stress, as well as their potential diagnostic use.

Methodology: We carried up cross-sectional analytical study with 60 participants, evenly divided between RA patients and healthy controls of the same age and gender. Malondialdehyde levels were measured spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) method, and isoprostanes were quantified using commercially available ELISA kits. The samples were obtained at the Arif Clinical and Diagnostic Centre and examined in the biochemical labs of Rashid Latif Hospital in Lahore.

Results: The study found that RA patients had significantly higher levels of MDA and isoprostanes in their serum (MDA: $1.95 \pm 0.094 \mu\text{mol/ml}$; Isoprostanes: $12.26 \pm 5.26 \text{ pg/ml}$) and synovial fluid (MDA: $3.26 \pm 0.65 \mu\text{mol/ml}$; Isoprostanes: $34.26 \pm 4.26 \text{ pg/ml}$) compared to controls (MDA: $0.95 \pm 0.019 \mu\text{mol/ml}$; Synovial Fluid MDA: 0.019 ± 0.0016).

Conclusion: Elevated levels of MDA and isoprostanes in RA patients highlight their significance as oxidative stress indicators, representing the continuous inflammatory process and probable joint damage in RA. These findings lend credence to the diagnostic and prognostic value of lipid peroxidation products in the treatment of RA.

Keywords: Rheumatoid Arthritis, oxidative stress, lipid peroxidation, malondialdehyde, isoprostanes.

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune, inflammatory illness that causes synovial hyperplasia, significant erosive destruction to the peripheral joints, and chronic inflammation of both small and large synovial joints.

Synovial cells proliferate characteristically, whereas macrophages, memory T lymphocytes, and plasma cells

are activated and infiltrated on a chronic basis. There is permanent loss of the joint bone and surrounding cartilage. [1,2] Leukotrienes, prostaglandins, proteases, complement system components, and a buildup of oxygen free radicals in the rheumatoid joint are among the inflammatory products that are produced. [3] With RA, there are extra-articular symptoms that affect the heart and lungs, as well as systemic vasculitis and progressive peripheral

neuropathy. Intestinal dystrophies, ischemic cardiovascular illnesses, renal glomerular failure, and pulmonary problems are among the primary causes of early mortality globally and are significantly increased in individuals with RA. Approximately 1% of people worldwide have a substantial prevalence of RA. [4] demonstrating the significant interaction between lifestyle, environmental, and genetic variables linked to the development and etiology of RA.

Finding these genetic and environmental variables linked to the onset of RA may be essential for early diagnosis and the implementation of preventative strategies.[5] Menstrual abnormalities, obesity, vitamin D insufficiency, alcoholism, and persistent smoking are risk factors linked to the development of RA.[6] Most significantly, an imbalance between pro- and antioxidant agents causes a marked rise in oxidative stress in the joints, which is crucial for the development and etiology of chronic disorders. There is adequate data from a number of research to conclude that highly reactive oxygen radicals in RA cause significant oxidative damage.[7] A negative correlation has been shown in several studies between the consumption of antioxidant-rich foods and the incidence of RA. [8] However, a small number of studies have shown that RA patients' synovial fluids and sera had higher oxidative enzyme activity and a lower quantity of antioxidants. Because of their extreme reactivity, it might be challenging to gauge an oxygen radical's concentration. As a result, it is possible to detect how these radicals' oxidative actions affect proteins, lipids, and DNA [9,10]. Patients with RA experience a higher incidence of atherosclerosis in medium-sized arterioles due to oxidative damage to membrane lipids. High levels of local and systemic inflammatory cytokines cause the surrounding lipids to break down, releasing free fatty acids (FFAs) that cause dyslipidemia and further harm to nearby tissues. Lipid peroxidation-induced DNA damage is notably seen in RA patients.[11] Reactive oxygen radicals and reactive nitrogen radicals can damage nucleotide codons irreversibly or induce DNA single strand breaks. Many end products are produced by lipid peroxidation, but two that are particularly significant are malondialdehyde (MDA) and isoprostanes, which have been discovered in higher amounts in the synovial fluids and sera of RA patients.[12]

This study's primary goal is to examine lipid peroxidation and pro-inflammatory biomarkers, particularly isoprostanes and malondialdehyde (MDA), in the synovial fluids and sera of patients with RA in order to comprehend their role in the genesis and development of the illness. Measuring these signs, relating them to clinical severity, and assessing their potential as predictive or diagnostic tools are among the objectives. The argument is predicated on the important role that oxidative stress plays in RA, where inflammation and cellular damage are caused by lipid peroxidation. By improving our knowledge of the molecular pathways underlying RA, our research aims to

alleviate the severe health burden associated with RA and perhaps improve diagnostic and therapy options.

MATERIALS AND METHODS

This cross-sectional study comprised 60 volunteers, split evenly into two groups: 30 patients with Rheumatoid Arthritis (RA) and 30 healthy controls of similar age and gender. Participants were recruited from the Orthopedics Department of Rashid Latif Hospital, Lahore. The study was conducted over a six-month period, following Research Ethical Approval certificate ref no. IRB-RLKU-032/05/02/2024 from the Research Ethics Committee of RLKU MEDICAL & DENTAL COLLEGE, Rashid Latif Khan University.

All participants provided informed consent prior to inclusion in the study. For each participant, 5 ml of venous blood was collected from the antecubital vein using standard venipuncture technique. Synovial fluid was obtained from patients undergoing routine arthrocentesis. Both types of samples were immediately processed: blood samples were centrifuged at 3000 rpm for 10 minutes to separate serum, and both serum and synovial fluid samples were then stored at -70°C until further analysis. Inclusion criteria encompass individuals aged 18 to 65, diagnosed with RA per American College of Rheumatology criteria, capable of providing informed consent, and undergoing routine arthrocentesis. Exclusion criteria include the presence of other autoimmune or chronic inflammatory diseases, recent use of steroids or immunosuppressive therapies, significant comorbid conditions such as advanced cardiac, renal, or hepatic diseases, pregnancy, and age outside the specified range. These criteria ensure a well-defined study population to accurately assess the impact of oxidative stress markers on RA.

Malondialdehyde (MDA) levels were determined using the method described by Ohkawa et al.[13] The experiment included combining 200µl of serum or synovial fluid with 8.1% sodium dodecyl sulfate (SDS), 1.5ml of 20% acetic acid (pH 3.5), and 1.5ml of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95°C for 60 minutes to form a thiobarbituric acid reactive substances (TBARS) complex. After cooling, 4ml of n-butanol was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The MDA concentration was calculated using a standard calibration curve. Isoprostane levels were tested using commercially available ELISA kits in accordance with the manufacturer's instructions. This approach quantified isoprostanes in serum and synovial fluid samples. The data were examined using SPSS software (version 27). Continuous variables were expressed as mean ± SD, and differences between RA patients and controls were analyzed using independent t-tests. Categorical variables were analyzed using the Chi-square test. A p-value < 0.05 was considered statistically significant. The Pearson

correlation coefficient was utilized to investigate the relationship between oxidative stress markers and RA clinical parameters.

RESULTS

Table 01 shows lipid peroxidation data that clearly demonstrate their participation in rheumatoid arthritis. The current study's findings show that MDA and isoprostane concentrations in blood and synovial fluid differed significantly between the two groups. In comparison to the control group, which had concentrations of 0.95 ± 0.019 , 0.056 ± 0.0056 , and 0.019 ± 0.0016 of MDA ($\mu\text{mol/ml}$) in serum and synovial fluid, RA patients had considerably higher quantities (1.95 ± 0.094 , 0.012 ± 0.0034 , and 3.26 ± 0.65) ($p < 0.015$, see table 01 and figure 01). The isoprostane concentrations in the RA participants were lower (12.26 ± 5.26 , 2.16 ± 0.019 , and 34.26 ± 4.26) than in the control group (1.26 ± 0.015 , 0.816 ± 0.017 , and 0.136 ± 0.019 , respectively). Figure 1 depicts the MDA levels in serum, which show a considerable rise in RA patients (about 1.95 nmol/L) compared to controls (0.95

nmol/L). Figure 2 depicts MDA levels in synovial fluid, where the difference is even more dramatic, with RA patients having levels of roughly 3.26 nmol/L compared to almost negligible quantities in controls. These findings clearly show that RA patients have higher oxidative stress, strengthening the usefulness of MDA as a diagnostic marker for Rheumatoid Arthritis and emphasizing the role of lipid peroxidation in the disease's progression. Furthermore, the figure shows MDA levels in saliva, which is an unusual medium for such measures in RA research, suggesting potential systemic oxidative stress beyond the regularly studied blood and synovial fluid. This might have broader implications for oxidative stress indicators in RA, indicating a larger field of diagnostic and therapeutic research. The higher MDA levels in all examined fluids indicate the ubiquitous nature of oxidative damage in RA, lending credence to the concept that regulating oxidative stress may play a critical role in reducing the severity and development of Rheumatoid Arthritis.

Table-1: Elevated Lipid Peroxidation Levels, Prominent Variable Expression of Diagnostic Significance, and Their Interaction in Rheumatoid Arthritis.

Variables	Controls (n=30)		Subjects (n=30)		P-value
	Serum	SynovialFluid	Serum	SynovialFluid	
MDA (nmol/ml)	0.95 ± 0.019	0.019 ± 0.0016	1.95 ± 0.094	3.26 ± 0.65	0.015
Isoprostanes (pg/ml)	1.26 ± 0.015	0.136 ± 0.019	12.26 ± 5.26	34.26 ± 4.26	0.022

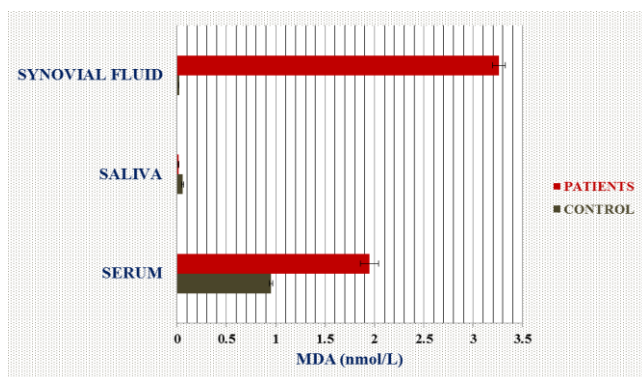


Figure 1: Levels Of Malondialdehyde (MDA) nmol/ml [P=0.015].

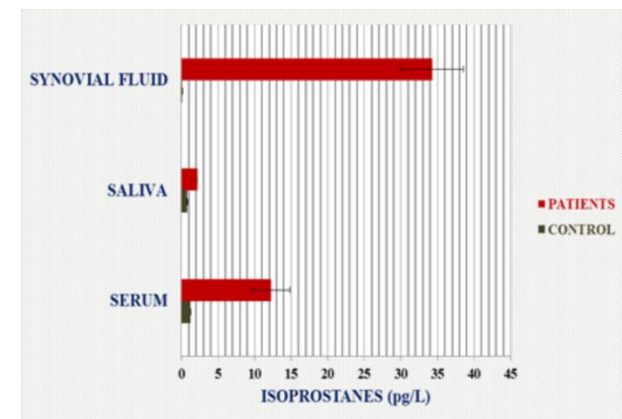


Figure 2: Levels Of Isoprostanes (pg/ml) [P=0.022].

DISCUSSION

In compared to healthy controls, people with Rheumatoid Arthritis (RA) showed significantly higher levels of isoprostanes and malondialdehyde (MDA) in their synovial fluids and blood, indicating severe oxidative stress. This is similar with earlier study finding that inflammatory byproducts are less concentrated in RA patients' blood, indicating a reduced antioxidant defence that raises the risk of reactive oxygen species (ROS)-induced tissue damage [14]. This incident is crucial because it highlights the link between high levels of oxidative stress and low antioxidant levels, which are common in persons with RA and osteoarthritis. This provides support to the hypothesis that nitrogen species and reactive oxygen play important roles in inflammatory responses. Increases in these reactive species can cause significant damage to matrix components, proteins, joint lipids, and DNA. In autoimmune disorders, the immune system of the body accidentally targets its own tissues, which is a common development [15].

Evidence suggests that oxidative damage to DNA, extracellular collagen, and cartilage is more severe in RA patients. Notably, investigations on RA patients' plasma showed a large increase in MDA levels and a corresponding drop in vitamin E [16, 17]. This includes queries performed by Lunec et al. The observed trend indicates increased levels of free radical activity, which can be exacerbated by external factors such as smoking

and chemicals that might alter DNA and impair replication and functioning [18, 19]. Furthermore, ROS have a role in both more widespread systemic effects and direct damage to cellular components.

By raising plasma levels of oxidized low-density lipoprotein and lipid peroxidation, they aid in the disease's rapid progression [20]. The synovial fluid's inflammatory cytokines, neutrophils, macrophages, and lymphocytes increase the generation of ROS and support the circumstances that prolong RA [21, 22].

Our findings on higher-than-normal levels of isoprostanes and HNE in synovial fluid and serum are consistent with the wider body of research, which highlights the importance of both enzymatic and non-enzymatic arachidonic acid oxidation in RA. When compared to healthy people, RA patients produce more lipid peroxidation end products, which can be related to the extensive oxidation process [23,24,25]. The stability of biological membranes is determined by interactions between lipids and proteins in biological systems. However, ROS has the ability to upset this equilibrium by modifying the fluidity of the membrane and triggering the release of cellular enzymes into the extracellular matrix, which is a unique feature of the cellular disruption observed in RA [26].

In summary, our findings underscore the critical role that oxidative stress plays in the genesis of RA, which has profound implications for our understanding of the molecular mechanisms that underpin the disease and potential therapy targets that may mitigate its repercussions.

CONCLUSION

Our research clarified the connection between inflammatory illnesses and oxidative stress. The current study's findings indicate that the generation of aldehydes, such as MDA, is correlated with the development of RA, suggesting the significance of lipid peroxidation in the pathophysiology and consequences of RA. One potential tool for tracking RA is the measurement of lipid peroxidation indicators in synovial fluid and serum. Thus, it is hypothesized that oxidative damage is essential to the development of RA.

Conflict of Interests: Authors declare no conflict of interests.

Authors' Contributions

US: conceptualized the study and supervised all phases of its execution.

MA: was responsible for the statistical analysis and manuscript preparation.

FM: managed the laboratory work, including sample processing and assay conduct.

SS: assisted in the study design and literature review.

SM: participated in data collection and entry.

JA: provided clinical insights, patient recruitment, and critical revisions of the manuscript. All authors read and

approved the final manuscript.

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