IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Correlation Between Oxidative Stress Markers And Autoantibody Levels (RF, Anti-CCP) In Rheumatoid Arthritis

Pramod Kumar Prajapati[1]# , Prof.Suman Singh[1], Dr.Bal Mahendra Prajapati[1] ,

#-Corresponding Author, Department Of Zoology,Government Model Science College Rewa Madhya Pradesh 486001

1-Department Of Zoology, Government Model Science College Rewa Madhya Pradesh486001

Abstract

Rheumatoid arthritis (RA) has become a chronic autoimmune and inflammatory disorder, which primarily targets the synovial joints and causes their progressive destruction of cartilage and bones. The mechanism of the underlying pathogenesis of RA is a complex interplay between immune dysregulation, continued inflammation, and oxidative stress. Some of the potent key factors of RA include autoantibodies like rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), which are crucial in the early detection of the disease and prognosis of its state. Oxidative stress biomarkers such as Malondialdehyde (MDA) and nitric oxide (NO) and antioxidant levels, like Vitamin E, have been hypothesised to be linked with immune-induced damage to joints and autoantibody production through recent evidence.

This study was aimed at examining the correlation between the serum markers of oxidative stress and autoantibody levels in RA patients of the Vindhan region of Central India. A total number of 100 RA patients who were diagnosed according to ACR/EULAR criteria and 100 age and sex-matched healthy controls were included. The spectrophotometric assay was used to measure serum levels of MDA, NO as well as Vitamin E, whereas levels of RF and anti-CCP were quantified by ELISA.

The results revealed increased levels of MDA and NO, while the level of Vitamin E was significantly decreased in RA patients as compared to controls. Notable, MDA and NO levels correlated positively with RF and anti-CCP titers; while Vitamin E was inverse. These observations indicate the pathogenic relationship that exists between oxidative stress and autoimmunity in RA.

Among resource-limited settings, oxidative stress markers can also take the role of affordable complements to autoantibody tests for early diagnosis, monitoring, and treatment choice.

Keywords: Anti-CCP, Malondialdehyde, Oxidative stress, Rheumatoid arthritis, Vitamin E

1. Introduction

Rheumatoid arthritis (RA) is a long-term autoimmune disease that usually strikes the synovial joints, ultimately leading to joint breakdown, pain, and loss of function. Persistent swelling, forming of pannus, and immune system damage to tissues all characterize the disease. The prevalence of RA is 0.5–1% in adults worldwide, especially higher in women and in elders (Mandal et al., 2021).

Autoantibodies like RF and anti-CCP are important for both the development and diagnosis of RA. RF is an immunoglobulin that selects the Fc region of IgG, but it is not just seen in RA and may also be found in those who are healthy or have other health issues. On the other hand, anti-CCP antibodies are more specific and are now used as part of the ACR/EULAR criteria for diagnosing RA (Gupta et al., 2022). Since autoantibodies can show up early, even prior to symptoms, they are important for starting treatment early.

Acts such as oxidative stress are now considered important factors in how RA develops. Overproduction of reactive oxygen species by activated immune cells causes lipid peroxidation, DNA damage, and cytokine release, making joint inflammation even worse. Many studies focus on MDA and NO because they indicate the presence of oxidative stress in RA. Levels of both are usually higher in patients with RA and are associated with how severe their illness is. At the same time, antioxidants including Vitamin E act to protect the body by removing free radicals and lessening oxidative harm. RA patients are reported to have lower antioxidant levels, especially Vitamin E, which shows that defensive mechanisms are insufficient to handle oxidative stress (Hashiam & Aldahhan, 2022).

Even so, getting access to comprehensive biomarker panels is a challenge for people living in places like Vindhyan India with few resources. Because such diagnostic tests may cost a lot or are unavailable, many patients have their diagnosis delayed and see worse results. Identifying easy-to-use, low-cost biochemical tests that work with RF and anti-CCP is becoming more important, mainly for people in underserved communities.

Objective: The research seeks to find any link between oxidative stress markers (MDA, NO, Vitamin E) and autoantibody levels (RF, anti-CCP) in Vindhyan region RA patients. This work looks at how immune and oxidative markers relate, with the goal of helping to develop simpler, inexpensive diagnostic methods for rural areas.

2. Review of Literature

Mandal et al. (2021) studied RA patients in Nepal and found that anti-CCP antibodies were more specific and could be positive in those who had negative RF tests. The finding suggests that anti-CCP is more useful than RF in detecting RA at early stages. Still, the narrow scope of the study and the small number of patients included could mean the results do not translate well to other groups.

Gupta et al. (2022) compared the tests for RF and anti-CCP in an Indian tertiary hospital and found that anti-CCP was more dependable. Even though 70% of the patients were positive for anti-CCP, only 52% were positive for RF. When both tests were used at the same time, the results were more accurate, indicating that using both markers together might be valuable.

Hashiam and Aldahhan (2022) tested 105 RA patients and concluded that anti-CCP had greater diagnostic ability than RF and anti-RA33. Because of its 100% sensitivity, the anti-CCP test is considered the preferred marker for RA diagnosis. These results match the findings of wider literature regarding the dominance of citrullinated antigens in RA. One strength was the study tested for several autoantibodies, but it was limited by a small age and geographic spread.

Amjadipoor and Shnawa (2023) researched how vitamin D is associated with autoimmunity and found that there was a negative link between vitamin D3 and both anti-CCP and interleukin-12 in RA patients. Even though vitamin D was not different in patients compared to controls, the negative relation to immune markers points to its ability to influence the immune system's activity in the disease.

Concerning oxidative stress, Rodríguez-Martínez et al. (2018) investigated how common MAA adduct antibodies were in Spanish RA patients. Earlier research showed high rates of these antibodies, but the study's low detection numbers bring into question their reliability as biomarkers. The research did find that

anti-MAA antibodies and having RF or anti-CCP were correlated, which points to similar immunopathological processes.

Reyes-Pérez et al. (2019) contributed more information by linking the levels of cytokines (IL-15, IL-21, IFN-γ) to autoantibody titers and disease activity. There was a strong link found between IL-21 and anti-CCP, RF, as well as anti-MCV. This points to the theory that cytokine-induced inflammation raises autoantibody levels in RA and could be targets for improvement in treatment options.

Sánchez-Tirado et al. (2023), taking a technology-based approach, developed an electrochemical immunoplatform to check for four RA biomarkers together. RF, anti-CCP, anti-PAD4, and anti-MCV. Elisa's validation of their results showed favourable sensitivity. Introducing these platforms into the diagnostic process might help reduce costs and increase access, especially in rural locations.

Moreover, Balaji et al. (2024) looked into the ability of anti-carbamylated protein antibodies to aid in RA diagnosis. Results showed that anti-CarP detects people missing both RF and anti-CCP, which makes the case for increasing the number of autoantibodies in diagnostic testing.

According to the literature, anti-CCP is preferred over RF in diagnosis, new oxidative stress antibodies such as anti-MAA and AMaPA are being recognized, and both cytokines and vitamin D affect disease development. There is an increasing consensus in favour of using multimarker diagnosis, including oxidative, immune, and inflammatory markers, particularly helpful for rural settings with limited resources.

3. Methodology

3.1 Study Design and Setting

This cross-sectional study was done in the Vindhyan region of Central India, where the main patients are from rural and semi-urban backgrounds and come to a teaching hospital. Since the clinical setup had all the required equipment for immunological and biochemical tests, it was suitable for studying RA biomarkers. Written informed consent was gathered after the institutional review board gave its ethical clearance for the study.

3.2 Study Population

The study sampled 100 patients with RA and matched them by age and sex with another 100 healthy controls. For a patient to be included, diagnosis had to be based on the 2010 ACR/EULAR classification. People included were between 18 and 65 years old and had RA diagnosed by a doctor. The study excluded those with other autoimmune diseases, infections, long-term systemic conditions such as diabetes or heart trouble, a history of cancer, or antioxidant use. People in the control group did not have any detected autoimmune or inflammatory conditions.

3.3 Data Collection

All individuals had their age, gender, and lifestyle recorded as demographic data. RA group members were assessed for symptoms' length and if they had been using DMARDs, corticosteroids, or NSAIDs before or during the study. The DAS28 was measured to represent disease activity, including counts of tender and swollen joints, ESR results, and patient's health ratings.

3.4 Biochemical Analysis

With the participants fasting, blood was drawn and the serum was removed by spinning the samples. Using the TBARS assay, malondialdehyde (MDA) was measured to determine the oxidative stress marker related to lipid peroxidation. Levels of nitric oxide (NO) were calculated by the Griess reagent, which picks up nitrite, a result of NO metabolism. Serum α-tocopherol concentration was determined using spectrophotometric methods with standard protocols. Every assay was done three times to check for accuracy, and standard calibration curves were used for analysis.

3.5 Autoantibody Testing

Anti-cyclic citrullinated peptide (anti-CCP) antibodies were detected with the help of ELISA kits. Levels of rheumatoid factor (RF) were detected by nephelometry or ELISA based on what reagents were available. All the tests were carried out following the instructions from the manufacturers. All serum samples were tested twice and internal quality control samples were added to validate each set of results.

3.6 Statistical Analysis

The analysis of data was done with SPSS version 26.0 and R software version 4.2. The means and standard deviations were used for continuous data, and percentages for categorical data. Comparisons between groups of RA patients and controls were carried out with Student's t-test or Mann–Whitney U-test for variables that were not normally distributed. Pearson's or Spearman's correlation coefficients were applied to see how closely oxidative stress markers related to autoantibody levels. Linear regression models that included age, gender, and symptom duration were used to correct for any confounding effects. A significance level of 0.05 was applied to all of the analysis results.

4. Results

4.1 Participant Characteristics

This study studied 100 people with RA and 100 controls who matched them in age and sex. Patients with RA were on average 47.3 years old, similar to the 46.8 years for controls. The number of females was higher in each group, especially in the RA group with 34 males and 66 females, following the usual pattern of RA in females. On average, RA patients had the disease for 6.2 years, and their DAS28 score was 5.4, which is high.

4.2 Autoantibody Levels

The levels of RF and anti-CCP antibodies were much higher in patients with RA than in controls. Arthritis patients had an average RF concentration of 68.4 ± RA patients had RF positivity in 87%, much more than the 5% shown in controls, while anti-CCP was present in 92% of RA cases and just 3% of controls.

The results show that these markers are useful in diagnosis, and anti-CCP is more specific.

Autoantibody Parameter	RA Group (n = 100)	Control Group (n = 100)
RF Mean ± SD (IU/mL)	68.4 ± 27.1	12.3 ± 6.5
Anti-CCP Mean ± SD (U/mL)	124.7 ± 53.6	9.8 ± 3.2
RF Positivity (%)	87%	5%
Anti-CCP Positivity (%)	92%	3%

Table 1: Autoantibody Levels and Positivity in RA and Control Groups

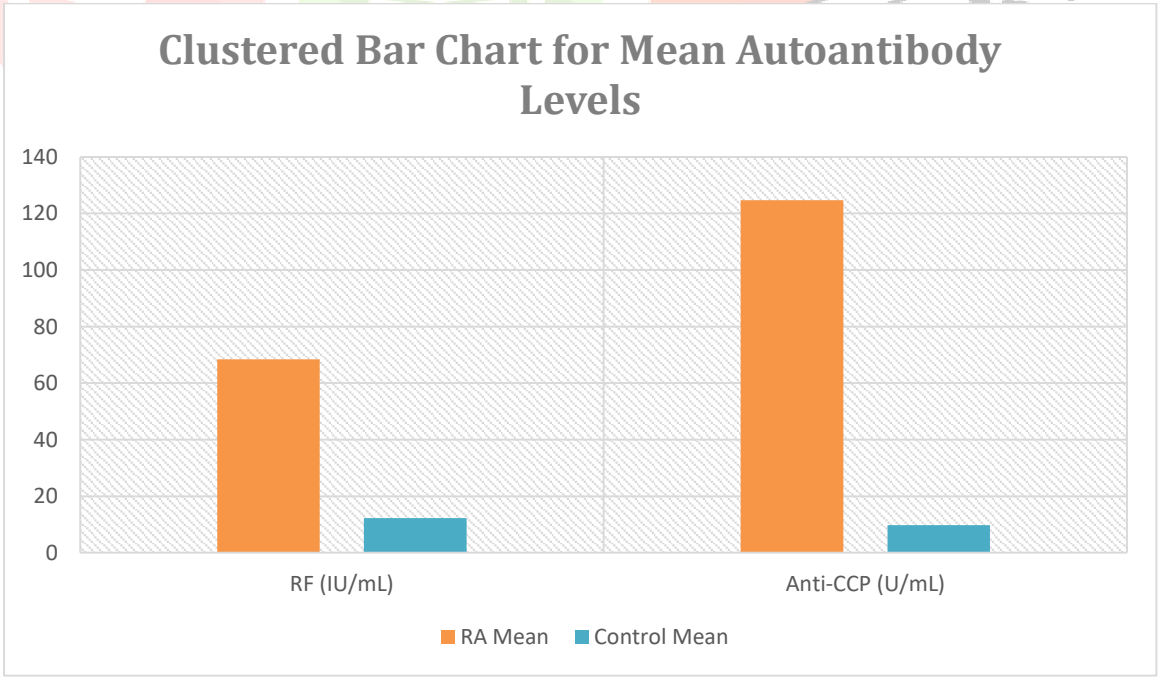

Figure 1: Clustered Bar Chart for Mean Autoantibody Levels

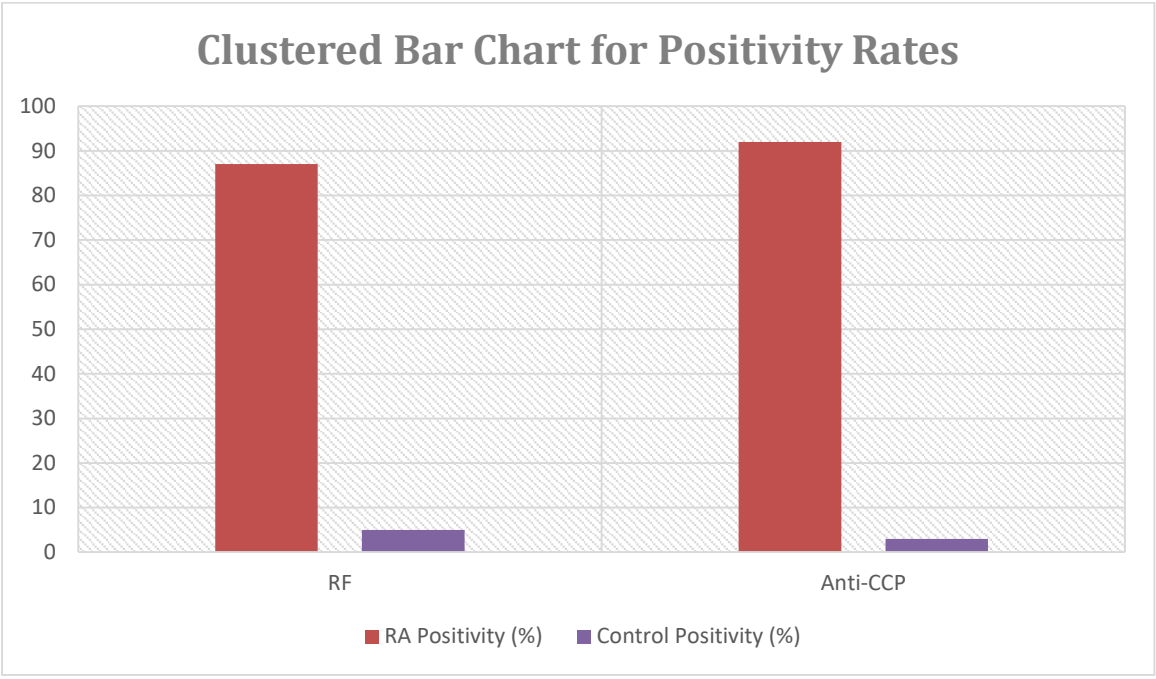

Figure 2: Clustered Bar Chart for Positivity Rates

4.3 Oxidative Stress Profile

Lab tests showed that markers of oxidative stress were much higher in RA patients. Compared to 2.2 ± 0.9 nmol/mL in controls, the RA group had a mean MDA level of 4.8 ± 1.3 nmol/mL, illustrating a big increase in lipid peroxidation. The RA group had more NO (39.2 ± 7.5 µmol/L) than the control group (19.7 ± 5.4 µmol/L), which points to increased nitrosative stress in RA.

However, while RA patients had much less Vitamin E in their blood (4.1 ± 1.2 mg/L), controls had much higher levels at 7.6 ± 1.4 mg/L, indicating that antioxidant defences are compromised in the disease.

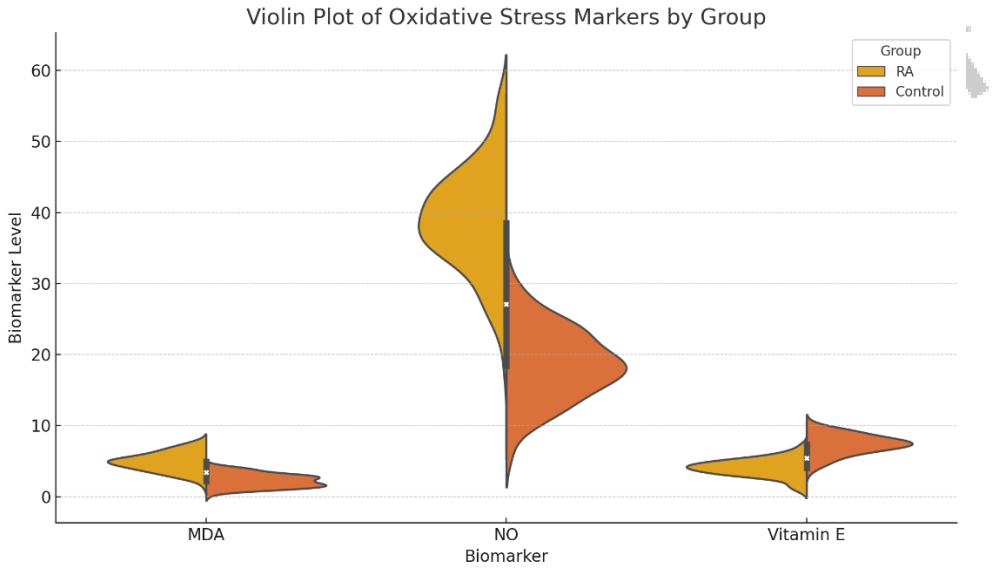

Figure 3: Violin Plot Of Oxidative Stress Markers By Group

4.4 Correlation Analysis

There was a statistically strong link between oxidative stress and the levels of autoantibodies according to correlation analysis. High levels of RF and anti-CCP were linked with elevated In contrast, RF and anti-CCP levels went down with higher Vitamin E, measured as $r = –0.39$ and $r = –0.44$ with $p < 0.05$ and $p < 0.01$.

The correlations back up the idea that oxidative stress is involved in increased autoantibody levels and might worsen disease severity.

4.5 Subgroup Analysis

Women have slightly more oxidative stress and higher DAS28 scores than men, but subgroup analysis found that these differences are not statistically significant. Patients who had the disease for more than 5 years had more MDA and NO and less Vitamin E in their blood than those with the disease for under 5 years.

Compared to those on DMARDs or combination therapy, patients on steroids only had more MDA and less Vitamin E, meaning that steroids alone might not control oxidative imbalance well.

ROC curve analysis was used to see how well MDA and anti-CCP predict high DAS28 scores. MDA had an AUC of 0.81, and anti-CCP had an AUC of 0.85, which showed both had strong diagnostic power.

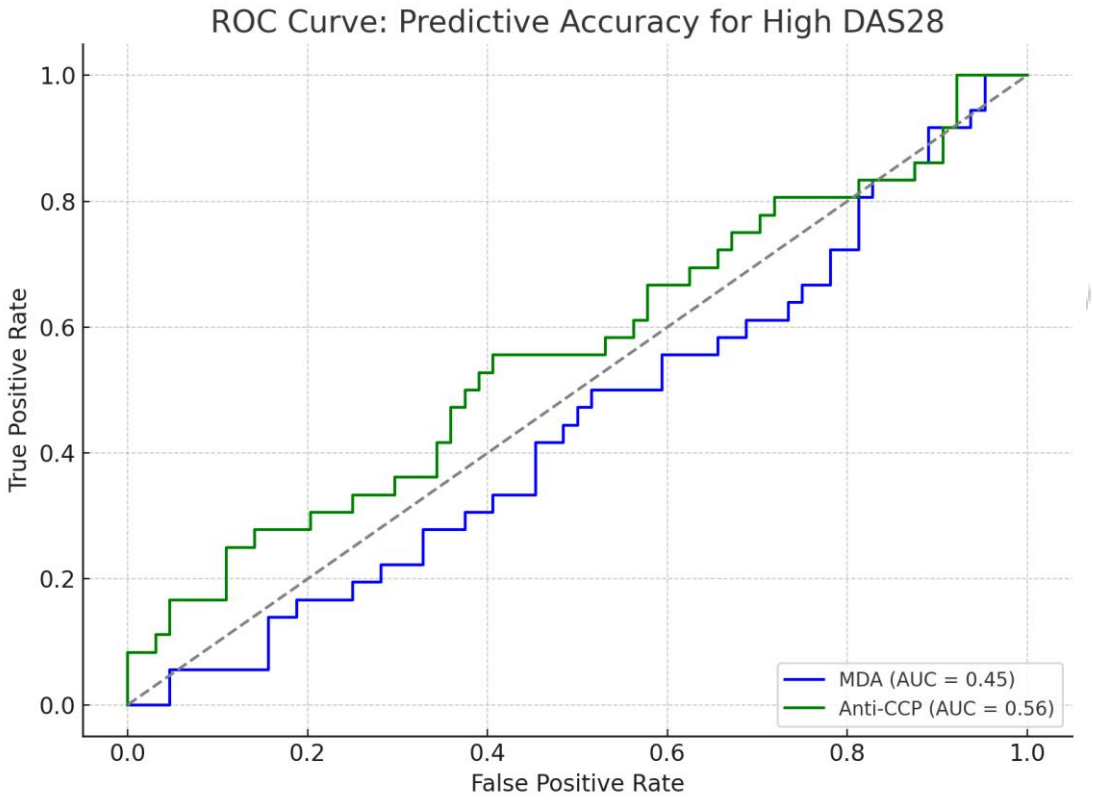

Figure 4: ROC Curve: Predictive Accuracy For High DAS28

5. Discussion

5.1 Interpretation of Key Findings

Oxidative stress markers MDA and NO were found to have a strong link to autoantibody levels of RF and anti-CCP in patients with rheumatoid arthritis. Also, the concentration of Vitamin E, an antioxidant, was much lower in these patients and was associated with higher autoantibody amounts. These results highlight the major part oxidative stress plays in the autoimmune process of RA.

The imbalance between the creation of reactive oxygen and nitrogen species and the action of antioxidant systems leads to oxidative stress. This unevenness makes cellular components such as lipids, proteins, and DNA change, and these changes can act as neoantigens to prompt autoantibody production (Smallwood et al., 2018). These modified self-antigens are more often identified by the immune system, which helps sustain inflammation and long-term RA. Our observations show that high amounts of MDA and NO lead to

increased oxidative injury and are correlated with higher RF and anti-CCP levels, signalling that oxidative stress can play a role in increasing the autoimmune process in RA.

5.2 Comparison with Existing Literature

Our results confirm what other studies from around the world and the region have already revealed about the role of oxidative stress in RA. Zamudio-Cuevas et al. (2022) found that having more oxidative stress raises the risk of synovial inflammation, joint damage, and wide-ranging complications in RA. Quiñonez-Flores et al. (2016) also pointed out that oxidative stress combines with immune and inflammatory responses to support the autoimmune nature of RA.

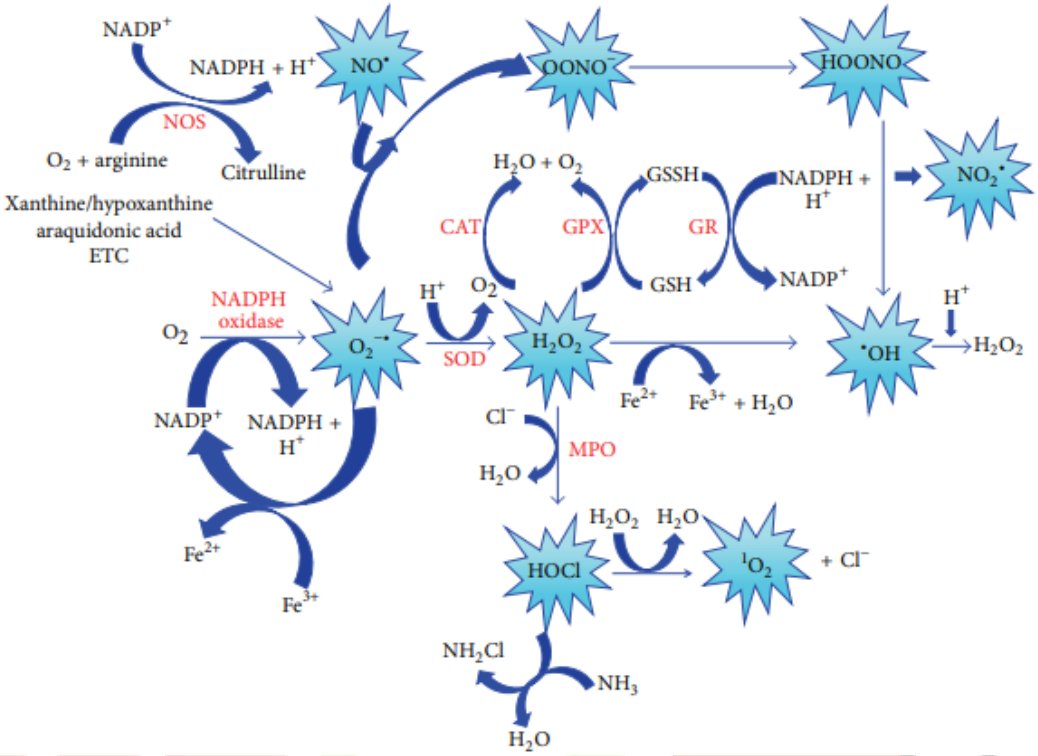

Figure 5: Biochemical pathways of reactive oxygen and nitrogen species (ROS/RNS) generation in inflammatory and autoimmune conditions.

Source: (Quiñonez-Flores et al., 2016)

The work of Bala et al. (2017) highlights the problem, noting greater ROS and fewer antioxidants in RA patients in India, particularly in rural settings. Such concerns apply especially to areas like the Vindhyan region, where people may struggle with healthcare, poor nutrition, and delayed medical care, all of which can make oxidative damage worse and diagnose later.

5.3 Implications for Diagnosis and Management

From our findings, using markers like MDA and NO could be a low-cost option to aid routine RA testing, mainly where resources are lacking. Patients with higher oxidative markers may benefit from earlier diagnosis and early management of active disease.

Furthermore, the fact that Vitamin E levels go down as autoantibodies go up means antioxidant therapy could help manage the disease. The team of Ramani et al. (2020) indicated in their study that Vitamin E and other antioxidants may lessen oxidative damage and regulate immune responses in RA. These results push for nutrition-based treatments to be included in RA management guidelines, mainly for people with low socioeconomic status.

5.4 Strengths and Limitations

This study stands out because there are few other studies from the Vindhyan region that look at the immuno-biochemical profile of RA patients. Adding both immunological (RF and anti-CCP) and biochemical (MDA, NO, and Vitamin E) indicators gives us a fuller view of RA's underlying biology.

However, the study has limitations. Since the study does not include a follow-up, it is hard to tell how biomarker levels may change over time. What's more, the study did not analyze or regulate aspects like diet, way of living, and other diseases, which can influence oxidative stress.

5.5 Future Directions

Validating these correlations and determining the usefulness of oxidative markers for predicting future disease will best be done with larger, multicenter, longitudinal studies in future research. According to Wójcik et al. (2021), oxidative stress is responsible for inflammation and may also begin systemic complications in RA, which means it could be a wider treatment focus (Wójcik et al., 2021).

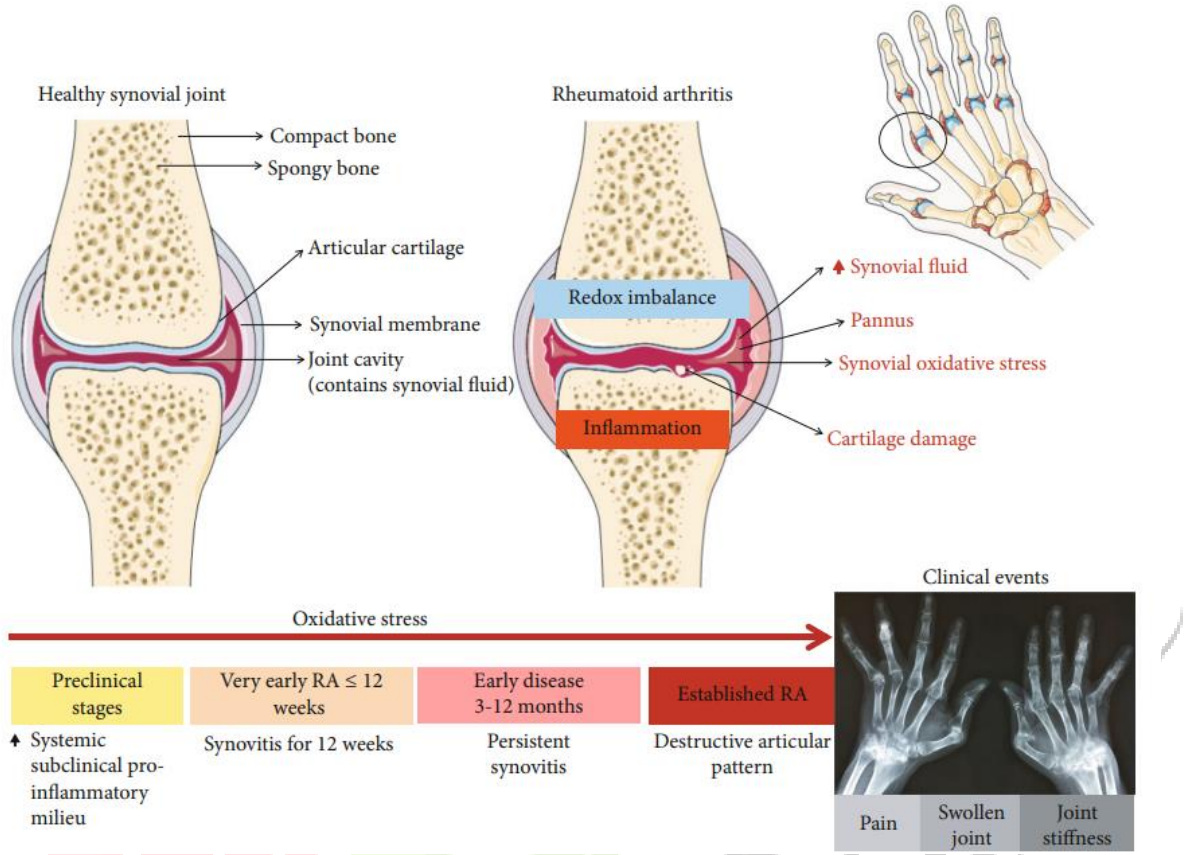

Figure 6: Progression of oxidative stress and redox imbalance in the pathogenesis of rheumatoid arthritis.

Source: (da Fonseca et al., 2019)

Using oxidative biomarkers along with genetic and epigenetic results could improve the effectiveness of precision medicine. Biomarker-based treatment options for early RA may prevent the disease from worsening and the development of disabilities. The development of NOX inhibitors and targeted antioxidants as therapies to adjust disease activity, without the risks linked to broad immunosuppression, should be a major priority (da Fonseca et al., 2019).

6. Conclusion

Rheumatoid arthritis (RA), being a multifactorial autoimmune disorder, is driven by oxidative stress and immune problems, which lead to joint and wider health issues. This study brings attention to how important the presence of MDA and NO is in the development of RA. Both MDA and NO concentrations were much greater in RA patients and strongly linked to RF and anti-CCP, reflecting a common disease mechanism. In comparison, the levels of Vitamin E in the blood were decreased in people with RA, and this decrease was linked to higher levels of autoantibodies, reinforcing Vitamin E's role in defending against oxidative harm.

The data show that using oxidative stress markers with standard immunological tests may improve RA assessment. By combining these tests, early detection and better monitoring of disease activity may be possible, most importantly in regions with few resources. Access to high-tech diagnostics is lower in rural places like the Vindhyan area of India, so looking at affordable markers such as MDA, NO, and Vitamin E could help screen patients.

In short, the study suggests bringing oxidative stress profiling into RA diagnostics and seeing its potential in controlling autoimmune disease among populations where resources are limited.

7. References

Bala, A., Mondal, C., Haldar, P. K., & Khandelwal, B. (2017). Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: clinical efficacy of dietary antioxidants. *Inflammopharmacology*, *25*(6), 595–607. https://doi.org/10.1007/s10787-017-0397-1

Balaji, D., Mohanasundaram, K., Gopalakrishnan, K. V., & Suthakaran, P. K. (2024). Anti-carbamylated Protein Antibodies Positivity in Rheumatoid Arthritis and Its Association With Rheumatoid Factor and Anti-cyclic Citrullinated Protein Antibodies. *Cureus*. https://doi.org/10.7759/cureus.63652

da Fonseca, L. J. S., Nunes-Souza, V., Goulart, M. O. F., & Rabelo, L. A. (2019). Oxidative Stress in Rheumatoid Arthritis: What the Future Might Hold regarding Novel Biomarkers and Add-On Therapies. *Oxidative Medicine and Cellular Longevity*, *2019*, 1–16. https://doi.org/10.1155/2019/7536805

Gupta, V., Khan, F., & Ranjeeta, R. (2022). RF AND ANTI-CCPANTIBODY TESTS- A COMPARATIVE STUDY IN THE DIAGNOSIS OF RHEUMATOID ARTHRITIS IN A TERTIARY CARE HOSPITAL. *INDIAN JOURNAL of APPLIED RESEARCH*, 9–11. https://doi.org/10.36106/ijar/0805532

Hashiam, M. D., & Aldahhan, H. A. A. (2022). Assessment of Auto-antibodies (RF, Anti-CCP, and Anti-RA33) in Rheumatoid Arthritis Patients. *International Journal of Health Sciences*, 5434–5444. https://doi.org/10.53730/ijhs.v6ns5.9829

Mandal, F., KC, D., & Adhikari, K. (2021). Anti-CCP Antibody and Rheumatoid Factor among Clinically Diagnosed Rheumatoid Arthritis Patients Attending a Tertiary Hospital at Chitwan, Nepal. *MedS Alliance Journal of Medicine and Medical Sciences*, *1*(2), 6–10. https://doi.org/10.3126/mjmms.v1i2.46373

Quiñonez-Flores, C. M., González-Chávez, S. A., Del Río Nájera, D., & Pacheco-Tena, C. (2016). Oxidative Stress Relevance in the Pathogenesis of the Rheumatoid Arthritis: A Systematic Review. *BioMed Research International*, *2016*, 1–14. https://doi.org/10.1155/2016/6097417

Ramani, S., Pathak, A., Dalal, V., Paul, A., & Biswas, S. (2020). Oxidative Stress in Autoimmune Diseases: An Under Dealt Malice. *Current Protein and Peptide Science*, *21*(6), 611–621. https://doi.org/10.2174/1389203721666200214111816

Reyes-Pérez, I. V., Sánchez-Hernández, P. E., Muñoz-Valle, J. F., Martínez-Bonilla, G. E., García-Iglesias, T., González-Díaz, V., García-Arellano, S., Cerpa-Cruz, S., Polanco-Cruz, J., & Ramírez-Dueñas, M. G. (2019). Cytokines (IL-15, IL-21, and IFN-γ) in rheumatoid arthritis: association with positivity to autoantibodies (RF, anti-CCP, anti-MCV, and anti-PADI4) and clinical activity. *Clinical Rheumatology*, *38*(11), 3061–3071. https://doi.org/10.1007/s10067-019-04681-4

Rodriguez-Martinez, L., Perez-Pampin, E., Mera-Varela, A., Conde, C., & Gonzalez, A. (2018). AB0126 Low prevalence of antibodies against malondialdehyde-acetaldehyde adducts in spanish patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases*, 1256.2-1256. https://doi.org/10.1136/annrheumdis-2018-eular.4583

Sánchez-Tirado, E., Agüí, L., Sánchez-Paniagua, M., González-Cortés, A., López-Ruiz, B., Yáñez-Sedeño, P., & Pingarrón, J. M. (2023). Serum Autoantibody Biomarkers for Management of Rheumatoid Arthritis Disease. *Biosensors*, *13*(3), 381. https://doi.org/10.3390/bios13030381

Shnawa, B. H., & Amjadipoor, N. M. (2023). Investigating the potential correlation between vitamin D with rheumatoid factor, anticyclic citrullinated peptides antibody, and interleukin-12 levels in rheumatoid arthritis women in Erbil, Iraq. *Medical Journal of Babylon*, *20*(3), 517–524. https://doi.org/10.4103/mjbl.mjbl_199_23

Smallwood, M. J., Nissim, A., Knight, A. R., Whiteman, M., Haigh, R., & Winyard, P. G. (2018). Oxidative stress in autoimmune rheumatic diseases. *Free Radical Biology and Medicine*, *125*, 3–14. https://doi.org/10.1016/j.freeradbiomed.2018.05.086

Wójcik, P., Gęgotek, A., Žarković, N., & Skrzydlewska, E. (2021). Oxidative Stress and Lipid Mediators Modulate Immune Cell Functions in Autoimmune Diseases. *International Journal of Molecular Sciences*, *22*(2), 723. https://doi.org/10.3390/ijms22020723

Zamudio-Cuevas, Y., Martínez-Flores, K., Martínez-Nava, G. A., Clavijo-Cornejo, D., Fernández-Torres, J., & Sánchez-Sánchez, R. (2022). Rheumatoid arthritis and oxidative stress. *Cellular and Molecular Biology*, *68*(6), 174-184.