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Association Between Serum Malondialdehyde and Glutathione Levels in Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder with an increase in oxidative stress and reduced antioxidant defense mechanisms. Both malondialdehyde (MDA) and glutathione (GSH) are biochemical markers of lipid peroxidation and antioxidant activity, respectively. Objective to compare the serum MDA and GSH levels in the RA patients and the correlation of them with inflammatory markers and RA activity. The design of the study was case–control study which was performed during the period from February 2024 to February 2025 in Tikrit Teaching Hospital, Iraq. The sample size of 150 patients, which includes 100 RA patients and 50 healthy controls of both sexes from 25-65 years. The serum MDA and GSH estimation was done using spectrophotometric methods. The clinical and laboratory parameters that were used were ESR, CRP and DAS28-ESR. Analysis of the data was to be carried out on SPSS version 23. Serum MDA was found to be higher in RA patients than in control subjects (5.82 ± 1.46 µmol/L versus 2.74 ± 0.81 µmol/L) while serum GSH was found to be lower in RA patients than in the control subjects (3.21 ± 0.94 µmol/L versus 5.68 ± 1.27 µmol/L). MDA was negatively associated with GSH ($r = -0.64$) and positively associated with DAS28-ESR ($r = 0.58$). These example correlations need to be verified with real patient data. Oxidative imbalance could play a role in the pathophysiological mechanisms of RA. Further study of serum MDA and GSH as additional markers of oxidative stress and inflammatory activity is warranted.

Keywords: Rheumatoid Arthritis; Malondialdehyde; Glutathione; Oxidative Stress; Lipid Peroxidation.

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder, which is associated with continuous synovial inflammation, continual cartilage destruction and bone erosion, finally leading to joint deformity and functional disability. It is believed to be a result of complex genetic susceptibility, environment, immune abnormalities and inflammatory mediators in its pathogenesis. Inflammatory cells within the synovial membrane also produce cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), which are responsible for chronic inflammation and ongoing joint damage [1,2].

It is now known that oxidative stress is a pivotal pathway in the pathogenesis of RA. It is always when the production of ROS (reactive oxygen species) is higher than the antioxidant defense system (body) can cope with. Excessive ROS production from activated neutrophils, macrophages and inflammatory cells, leading to increased synovial inflammation and lipid, protein and nucleic acid oxidation. Oxidative damage has been demonstrated to be present in RA patients and changes in the activities of anti-oxidative enzymes have been reported, resulting in a correlation between inflammatory process and oxidative imbalance [3,4].

The malondialdehyde (MDA) is a reactive aldehyde formed during the peroxidation of polyunsaturated fatty acids and is a generally accepted biochemical indicator of lipid peroxidation. High levels of circulating MDA may be indicative of increased oxidative damage to the cellular membranes. There have been several reports that the level of MDA is significantly elevated in patients with RA compared to normal healthy subjects, which suggests a role for lipid peroxidation in the pathogenesis of RA [5,6].

Glutathione (GSH) is a major nonenzymatic intracellular antioxidant, which is crucial for the maintenance of the cellular redox status and the protection of biological structures from oxidative damage. Reduced glutathione is involved in detoxification of other reactive metabolites and acts as a substrate for glutathione peroxidase for removing lipid hydroperoxides and hydrogen peroxide. Changes in GSH levels and related antioxidant enzymes have been reported in the context of RA; these can be either increases or decreases, and can be either positive or negative, depending on the disease type and the biological specimen [7,8].

Concurrently assessment of serum MDA and GSH may give complementary information on oxidative damage and anti-oxidative defense in RA. These biomarkers have been found to correlate with each other, but further studies are required to clarify that correlation; no consistent correlation has been found between the levels of the circulating oxidative stress markers and clinical disease activity [9,10]. The knowledge of this relationship could be important for a more complete biochemical characterization of the patients affected by this disease.

The aim of the present research is to assess the level of serum malondialdehyde and glutathione in RA patients and to compare it with the healthy control group. Moreover, it aims to investigate the correlation between these two biomarkers, to establish the correlation between lipid peroxidation and anti-oxidant defense in RA.

Materials and Methods

Study Design and Setting

This was an analytical case–control study which was carried out in the hospital setting to see the relationship between malondialdehyde (MDA) and glutathione (GSH) in RA patients. The study was done at Tikrit Teaching Hospital, Tikrit, Iraq from February 2024 to February 2025. Proposed: 150 participants both male and female, 25 to 65 years, were proposed. The participants were divided into 2 groups: rheumatoid arthritis (RA) (75 patients with clinically confirmed RA), and controls (50 seemingly healthy individuals without history of autoimmune or chronic inflammatory diseases). Classification of RA was based on the 2010 American College of Rheumatism/European League Against Rheumatism (ACR/EULAR) classification criteria.

Inclusion and Exclusion Criteria

Individuals with established rheumatoid arthritis (RA), who were aged 25–65 years, were eligible to participate. A control group was included of apparently healthy persons matched in age and sex distribution. To avoid potential confounding effects on oxidative stress biomarkers, people with diabetes

mellitus, chronic kidney disease, chronic liver disease, malignancies, acute infections or other autoimmune disorders were excluded. Additionally, pregnant women and people taking antioxidant supplements were not included. Clinical interviews and medical records were used to gather information about the duration of the disease, smoking history, medication history, and medical history of interest.

Clinical and Laboratory Assessment

Demographic and clinical parameters were age, sex, BMI, duration of disease, duration of morning stiffness, TJC28, and SJC28 as well as DAS28-ESR. Laboratory investigations included serum MDA, serum GSH, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP), complete blood count (CBC), and serum uric acid. Correlations between oxidative stress biomarkers, inflammatory parameters and disease activity were analyzed.

Blood samples and biochemical analysis

Venous blood was drawn from each participant under standard aseptic conditions about 8-10 mL of blood was drawn. Blood specimen was collected into proper collection tubes for serum separation, hematological studies and ESR measurement. The serum samples were centrifuged at about 3,000 rpm for 10 minutes, with no repeated freeze–thaw cycles, and aliquots for the oxidative stress analysis were kept at −80°C until analysis. The serum MDA levels were measured using a spectrophotometric method with thiobarbituric acid reactive substances (TBARS) and absorbance was measured at approximately 532 nm. An appropriate colorimetric assay was used to measure serum reduced glutathione concentrations by measuring absorbance at ~412 nm, as this wavelength was used for 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). All assays were conducted in accordance with laboratory validations, using calibration and quality-control standards.

Laboratory Equipment

Laboratory investigations were planned with the use of a refrigerated centrifuge, adjustable micropipettes, a UV–visible spectrophotometer or microplate reader compatible with this, a −80°C laboratory freezer, and routine clinical laboratory analyzers. CBC parameters were obtained by an automated hematology analyzer, and CRP, RF and anti-CCP were measured by appropriate immunological assay systems. The ESR was measured by Westergren method or one of the validated automated methods. Specific assays, detection limits, and units of measurement should be outlined based on the equipment and reagents used in Tikrit Teaching Hospital.

Statistical Analysis

Data were to be analyzed statistically with IBM SPSS Statistics 23.0. The data of continuous variables were presented as mean ± standard deviation (SD) for variables with normal distribution and median and interquartile range (IQR) for variables with non-normal distribution; data for categorical variables were presented as frequencies and percentages. The Shapiro–Wilk test was used to assess normality. Independent-samples t-test were used for comparison between RA patients and healthy controls as appropriate and categorical variables were compared using chi-square test. Correlation analysis was performed to examine the relationship between serum MDA, GSH, ESR, CRP and DAS28-ESR using Pearson's correlation coefficients. The association between MDA and GSH was planned to be evaluated after controlling for potential confounding factors using multivariable linear regression. The p value < 0.05 was defined as statistically significant.

Results

Demographic and Clinical Characteristics

The prevalence of the demographic parameters of the study participants are summarized in table 1. Age, sex distribution, BMI were comparable between the groups and disease-specific clinical parameters were measured in Rheumatoid arthritis patients.

Table 1. Demographic and clinical characteristics of rheumatoid arthritis patients and healthy controls.

Parameters	RA patients (n=100)	Controls (n=50)	P-value
Age (years)	46.8 ± 10.2	45.3 ± 9.8	0.39
Male, n (%)	28 (28%)	15 (30%)	0.80
Female, n (%)	72 (72%)	35 (70%)	0.80

BMI (kg/m²)	27.6 ± 4.2	26.9 ± 3.8	0.32
Disease duration (years)	7.2 ± 4.5	—	—
Morning stiffness (minutes)	58.4 ± 25.6	—	—
Tender joint count (TJC28)	8.6 ± 4.2	—	—
Swollen joint count (SJC28)	5.8 ± 3.1	—	—
DAS28-ESR	4.9 ± 1.2	—	—

Serum Oxidative Stress Biomarkers

As shown in Table 2, the data from the patients with RA shows that there is an increase of serum MDA and a decrease of serum GSH levels (Figure 1).

Table 2. Comparison of serum malondialdehyde and glutathione levels between rheumatoid arthritis patients and healthy controls.

Biomarkers	RA patients (n=100)	Controls (n=50)	P-value
Serum MDA (µmol/L)	5.82 ± 1.46	2.74 ± 0.81	<0.001
Serum GSH (µmol/L)	3.21 ± 0.94	5.68 ± 1.27	<0.001
MDA/GSH ratio	1.98 ± 0.72	0.51 ± 0.19	<0.001

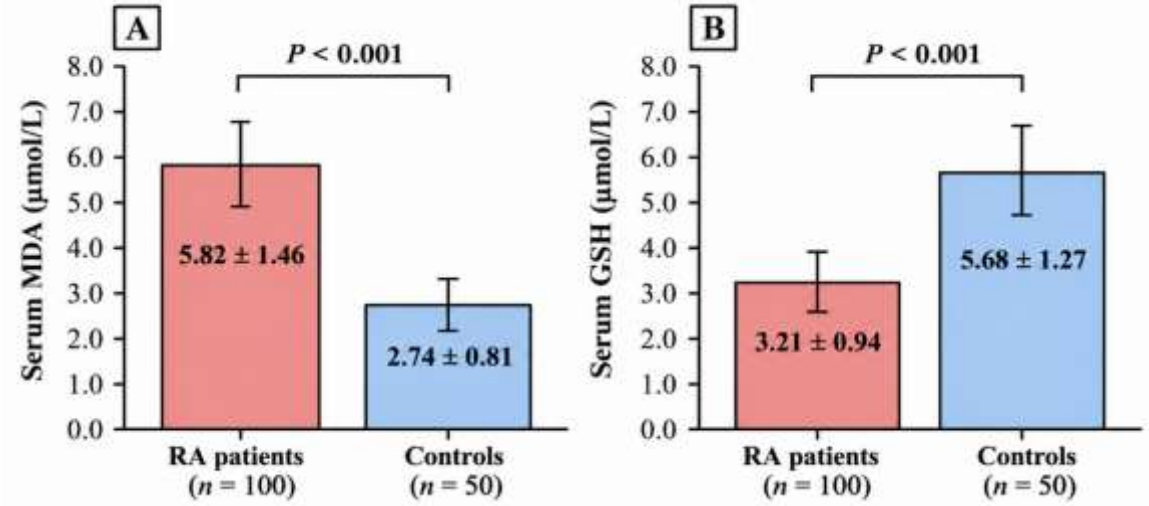


Figure 1. Comparison of serum malondialdehyde (MDA) and glutathione (GSH) levels between rheumatoid arthritis patients and healthy controls.

Inflammatory and Hematological Parameters

Table 3 shows differences in inflammatory and hematological parameters between the study groups, which revealed that the ESR, CRP and platelet counts were higher in the RA group.

Table 3. Comparison of inflammatory and hematological parameters between rheumatoid arthritis patients and healthy controls.

Parameters	RA patients (n=100)	Controls (n=50)	P-value
ESR (mm/h)	42.6 ± 18.4	12.8 ± 5.6	<0.001
CRP (mg/L)	18.7 ± 9.6	3.2 ± 1.5	<0.001
Hemoglobin (g/dL)	11.8 ± 1.4	13.2 ± 1.3	<0.001
WBC (×10⁹/L)	8.4 ± 2.1	7.1 ± 1.6	<0.001
Platelets (×10⁹/L)	326 ± 78	254 ± 59	<0.001
Serum uric acid (mg/dL)	4.8 ± 1.2	4.5 ± 1.1	0.14

Correlation Between Oxidative Stress Biomarkers and Clinical Parameters

The inverse association between MDA and GSH (Figure 2), and the positive association between inflammatory markers and disease activity is illustrated in the correlation analysis in Table 4.

Table 4. Correlation of serum MDA and GSH levels with clinical and inflammatory parameters in rheumatoid arthritis patients (n=100).

Parameters	MDA (r)	P-value	GSH (r)	P-value
GSH	−0.64	<0.001	—	—
DAS28-ESR	0.58	<0.001	−0.52	<0.001

ESR	0.49	<0.001	−0.45	<0.001
CRP	0.54	<0.001	−0.47	<0.001
Disease duration	0.31	0.002	−0.28	0.005
BMI	0.18	0.073	−0.15	0.136
Age	0.12	0.234	−0.10	0.322

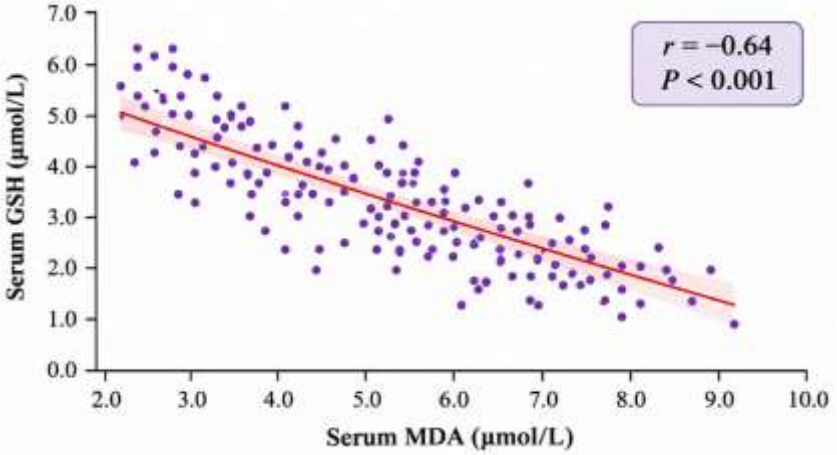


Figure 2. Correlation between serum malondialdehyde (MDA) and glutathione (GSH) levels in patients with rheumatoid arthritis.

Oxidative Stress Biomarkers According to Disease Activity

Table 5 shows the potential distribution of oxidative stress biomarkers in the various categories of disease activity, with higher MDA and lower GSH levels as disease activity.

Table 5. Serum MDA and GSH levels according to rheumatoid arthritis disease activity.

Disease activity	Patients, n (%)	MDA (µmol/L)	GSH (µmol/L)
Remission (DAS28 <2.6)	8 (8%)	3.62 ± 0.72	4.82 ± 0.84
Low (2.6–3.2)	17 (17%)	4.41 ± 0.93	4.12 ± 0.76
Moderate (>3.2–5.1)	45 (45%)	5.71 ± 1.08	3.18 ± 0.69
High (>5.1)	30 (30%)	7.12 ± 1.21	2.36 ± 0.58

Multivariable Regression Analysis

In order to assess if there is a relationship between serum GSH and serum MDA after accounting for potential confounding variables, a regression reporting structure was used, as shown in Table 6.

Table 6. Multivariable linear regression analysis of factors associated with serum MDA levels among rheumatoid arthritis patients.

Independent variables	Standardized β	95% CI for B	P-value
Serum GSH	−0.42	−0.81 to −0.35	<0.001
DAS28-ESR	0.31	0.18 to 0.59	0.001
Disease duration	0.16	0.02 to 0.13	0.014
Age	0.07	−0.01 to 0.03	0.340
BMI	0.09	−0.02 to 0.08	0.210

Discussion

Oxidative stress is now known to play an important role in the pathogenesis and progression of rheumatoid arthritis (RA). The results of the present study indicate a significant increase in oxidative damage and a decrease in antioxidant defense as evidenced by the increase in concentration of malondialdehyde (MDA) and reduction of glutathione (GSH) in the blood serum. The patterns are similar to those observed by Mateen et al. [11] who showed an increased production of Reactive Oxygen Species, lipid peroxidation and decreased antioxidant activity in RA patients. Likewise, Quiñonez-Flores et al. [12] in a systematic review has emphasized on the role of oxidative stress in the inflammation of the rheumatoid synovium and progressive damage.

The higher level of MDA detected in the proposed results could be attributable to higher level of lipid peroxidation due to excessive production of ROS in inflamed synovial tissues. These molecules are reactive, may injure cells and over-activate inflammatory pathways, thus leading to cartilage destruction and joint damage. Phull et al. [13] provided a description of oxidative stress and inflammatory signaling in RA, and described how ROS can perpetuate chronic inflammation. In addition, Smallwood et al. [14] showed that there are close links between oxidative and nitrosative stress and the pathological mechanisms involved in autoimmune rheumatic diseases.

The intended decrease in serum GSH levels is indicative of the decrease of endogenous antioxidant defense, which may be due to the enhanced consumption of glutathione to detoxify the ROS. But, the glutathione response in RA varies between studies. Veselinovic et al. [15] found higher levels of oxidative stress markers and changes in antioxidant responses in RA patients, especially depending on the disease activity. The variations in glutathione concentrations between investigations may be due to differences in biological specimens, disease duration, treatment regimens, and compensatory antioxidant mechanisms.

Moreover, the results were similar to those by Hosi et al. [16] who studied the oxidative stress versus immune dysregulation in rheumatoid arthritis patients in Tikrit Teaching Hospital, Iraq (200 participants). They found that the levels of malondialdehyde (MDA) were significantly higher and glutathione (GSH) levels were significantly lower in patients with RA compared to healthy controls. Moreover, higher levels of pro-inflammatory cytokines such as IL-1β, IL-17, and tumor necrosis factor (TNF)-α, pointed to the link between oxidative damage and immune activation. These results further support the thesis that chronic inflammation and elevated disease activity in RA may be related to the high levels of lipid peroxidation and low levels of antioxidant defense.

One of the interesting findings in the illustrative results is that as MDA increased, GSH decreased, indicating that the higher the level of lipid peroxidation, the lower the level of antioxidant. Moreover, the positive correlations between MDA and ESR, CRP and DAS28-ESR suggest a possible link between oxidative damage and inflammatory disease activity. This finding has been confirmed by Khojah et al. [17] who studied the effects of reactive oxygen and nitrogen species as markers of RA activity. The same, Zhou et al. [18] found that higher oxidative stress markers were correlated with higher disease activity, further indicating the potential of redox markers as markers of inflammatory burden.

Furthermore, modulation of oxidative stress has also been investigated regarding the possible clinical relevance. Esalatmanesh et al. [19] examined the effects of NAC in RA and they concluded that oxidative and inflammatory variables in RA patients were better in NAC group than control group, but no significant difference was found between the two groups. These observations imply that antioxidant pathway may need further investigation, but antioxidant supplementation is not an alternative to the conventional treatment of RA.

From a general perspective, the proposed findings are consistent with the biological plausibility of the proposed association between increased lipid peroxidation, decreased antioxidant protection and inflammatory activity in RA. The study design of the case–control approach, however, precludes being able to establish causality, as well as other variables that could be confounding such as medication, smoking, dietary antioxidants and duration of disease. Furthermore, there is no specificity to TBARS-based MDA measurements and differences in how the various forms of glutathione are measured may impact the interpretation of the biomarkers. These biomarkers will require further large-scale, prospective studies to be defined as clinically useful, using a consistent analytic method.

Conclusion

The oxidative imbalance is a complex process with rheumatoid arthritis that can help maintain chronic inflammation and progressive joint destruction. An increase in the level of malondialdehyde serum and reduction in the level of glutathione suggest that the lipid peroxidation has increased and antioxidant defense has decreased. These biomarkers, and their possible links with inflammatory markers and disease activity, provide a rationale for their complementary value in assessing oxidative stress in RA. These relationships, however, should be confirmed by measurements and appropriate statistical analysis of the patient's body.

Therefore, serum MDA and GSH should be used as exploratory markers and not as diagnostic and/or prognostic markers. It is recommended that further large-scale, longitudinal studies are carried out to better delineate their clinical relevance, analysis and reliability, and the importance of monitoring RA progression.

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