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# Evaluation of Oxidative Stress and Inflammatory biomarkers in Iraqi Patients with Rheumatoid Arthritis

## Oxidative & Inflammatory Markers in RA

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**ABSTRACT:** Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease in which oxidative stress and inflammation are closely linked, yet integrated evaluation of these processes in Iraqi patients remains limited. This cross-sectional case-control study included 80 adults in Baghdad, Iraq: 40 patients fulfilling the 2010 ACR/EULAR classification criteria and 40 age- and sex-matched healthy controls. Serum malondialdehyde (MDA), glutathione (GSH), total antioxidant capacity (TAC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) were measured. Between-group differences were evaluated with independent-samples t-tests, with Mann–Whitney U sensitivity analyses for selected markers, and pooled Pearson correlations were assessed using a Bonferroni-adjusted significance threshold. All seven biomarkers differed significantly between groups (all $p < 0.001$): MDA, TNF-α, IL-6, CRP, and ESR were higher in RA, whereas GSH and TAC were lower. Standardized mean differences were very large (Cohen's $d = 2.96–4.26$). Pooled correlations showed positive associations among oxidative and inflammatory markers and inverse associations with antioxidant markers, although these coefficients may partly reflect the strong separation between cases and controls. Exploratory rank-based study-group discrimination was also high, but the healthy-control case-control design and extreme biomarker separation limit interpretation as clinical diagnostic accuracy. A parsimonious two-predictor model containing MDA and TNF-α showed strong in-sample association with case status but was not treated as a validated prediction model. These findings support concurrent redox and inflammatory abnormalities in Iraqi RA patients. Larger studies with clinical comparator groups, participant-level validation, and longitudinal follow-up are required before diagnostic or monitoring applications can be inferred.

## 1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by symmetric polyarthritis, progressive joint destruction, and substantial extra-articular morbidity, affecting approximately 0.5–1% of adults worldwide [1,2]; one of the few population-based surveys conducted in Iraq estimated a prevalence of approximately 1% [42]. The diagnosis is based

on the 2010 ACR/EULAR classification criteria, together with clinical evaluation and serological tests [3] and untreated disease is associated with increased cardiovascular and disability risks [4,5].

Oxidative stress and pro-inflammatory cytokine signaling are interdependent and play a role in the pathogenesis of RA. Synovial macrophages and fibroblast-like synoviocytes produce reactive oxygen species (ROS) which can harm lipids, proteins and DNA [6–8,32] and cytokines (TNF-α and IL-6) activate synovial inflammation and cartilage degradation [6–8]. Clinical markers of systemic inflammation such as the acute-phase reactants CRP and ESR remain the most commonly used but these do not specifically reflect the oxidative component of disease activity [10].

Malondialdehyde (MDA) is an end product of lipid peroxidation and is one of the most well-researched oxidative stress markers in RA and has been found to be increased in various groups of RA patients [11–13]. Concurrently, antioxidant levels (glutathione (GSH) and total antioxidant capacity (TAC) are reduced, when ROS generation exceeds removal [14,15]. There are a number of groups who have suggested that a combination of oxidative-stress and inflammatory markers would be better for diagnostic and monitoring purposes than either domain alone [11,16,17].

Iraq provides an important context for RA biomarker research. Genetic polymorphisms in inflammatory-pathway genes, including NLRP3 inflammasome components, have been associated with RA susceptibility and treatment response in Iraqi patients [19]. Iraqi studies have also evaluated serological, oxidative, and inflammatory markers in RA [20–22,36,39], including concurrent assessment of MDA, GSH, and pro-inflammatory cytokines [36] and TNF-α/CRP/ESR in relation to disease activity [39]. However, to our knowledge, no previous Iraqi study has evaluated this specific seven-analyte panel (MDA, GSH, TAC, CRP, ESR, TNF-α, and IL-6) within the same cohort while jointly describing between-group effect sizes, pooled correlation patterns, and exploratory study-group discrimination. Because comparisons between established RA cases and healthy controls can exaggerate apparent classification performance, any discrimination analyses in the present study were interpreted as exploratory rather than as evidence of clinical diagnostic accuracy.

This study therefore aimed to (i) compare serum MDA, GSH, TAC, CRP, ESR, TNF-α, and IL-6 between Iraqi RA patients and matched healthy controls; (ii) explore pooled correlation patterns among these biomarkers while recognizing the potential influence of case-control group separation; and (iii) describe exploratory study-group discrimination and a parsimonious two-marker association with RA case status without inferring validated clinical diagnostic or monitoring performance.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Setting

The study design was a cross-sectional, case-control study that lasted from February 2025 to July 2026 during which the participants were recruited on a consecutive basis to participate in the study and all the participants signed a written informed consent. The research was approved by [name of ethics committee/institution and ethics approval number]. The reporting is done according to the STROBE reporting guidelines for Observational Studies [23].

### 2.2 Participants

Ninety (90) healthy controls were recruited consecutively from hospital staff and community volunteers at the same facilities as the patients and matched for age and sex with the patients, and eighty (80) patients with RA who met a pre-specified DAS28-ESR cut-off of ≥ 3.2 at enrollment (and who did fulfill the 2010 ACR/EULAR classification criteria [24]). Because controls were drawn from staff and community sources rather than from the same referral pathway as cases, a health-volunteer selection effect cannot be excluded. Exclusion criteria for both groups included other autoimmune or chronic inflammatory disease, active infection, malignancy, pregnancy, use of antioxidant supplements within the preceding month, and treatment with biologic DMARDs. RA patients receiving conventional synthetic DMARDs (72.5%) and/or corticosteroids (45.0%) remained eligible; medication status was recorded but was not used as an exclusion criterion.

### 2.3 Sample Size

Sample size was estimated a priori using G*Power 3 [35], based on an anticipated effect size of d = 0.9 for the primary oxidative-stress comparison, α = 0.05, and power = 0.80, yielding a minimum of 21 participants per group. The target of 40 participants per group was selected to improve precision for secondary exploratory analyses; it was not designed to support development of a high-dimensional prediction model. The observed standardized between-group effects were substantially larger than the planning assumption and should therefore be interpreted cautiously and validated in independent cohorts.

### 2.4 Biochemical Measurements

Venous blood was drawn after an overnight fast. Serum MDA was measured using the thiobarbituric acid reactive substances (TBARS) assay; GSH by the Ellman colorimetric method; and TAC using the Cayman Chemical Antioxidant Assay Kit (Item No. 709001), which measures inhibition of metmyoglobin-catalyzed ABTS oxidation relative to Trolox and reports antioxidant capacity in Trolox equivalents. CRP was quantified by nephelometry, ESR by the Westergren method, and TNF-α and IL-6 by sandwich ELISA (R&D Systems). All assays were performed in duplicate by technicians blinded to case/control status. Assay-specific intra- and inter-assay coefficients of variation should be entered from verified laboratory quality-control records before submission: [insert verified QC values].

### 2.5 Statistical Analysis

Between-group comparisons used independent-samples t-tests for all seven biomarkers, reporting mean differences with 95% confidence intervals and Cohen's d as a standardized effect size. Distributional assumptions were assessed with the Shapiro–Wilk test; Mann–Whitney U tests were used as sensitivity analyses for GSH, TAC, and CRP and as confirmatory analyses for the remaining biomarkers. Pooled Pearson correlation coefficients among the seven biomarkers were calculated across all 80 participants with a Bonferroni-adjusted significance threshold for 21 pairwise comparisons ($\alpha = 0.05/21 = 0.0024$). Because group membership can itself induce correlations when biomarkers differ strongly between cases and controls, these pooled correlations were interpreted descriptively rather than as within-RA mechanistic associations. Exploratory study-group discrimination was summarized using rank-derived AUC values calculated from the directional Mann–Whitney statistic as $AUC = U/(nRA \times nControl)$, with direction reversed for biomarkers that were lower in RA. Reported cut-offs, sensitivity, and specificity were treated as descriptive operating points; empirical AUC confidence intervals and DeLong pairwise comparisons were not reported. A parsimonious exploratory logistic model containing MDA and TNF-α was summarized using coefficients, odds ratios with 95% confidence intervals, Nagelkerke $R^2$, and apparent classification accuracy. No stepwise variable-selection, bootstrap-optimism, or cross-validation performance claim was retained. Statistical significance was set at $p < 0.05$ unless otherwise Bonferroni-adjusted. Analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

## 3. RESULTS

### 3.1 Baseline Characteristics

RA patients and controls were well matched for age and sex (Table 1). Thirty of 40 RA patients (75.0%) were female. Twenty-nine (72.5%) RA patients were receiving conventional synthetic DMARDs and 18 (45.0%) were receiving oral corticosteroids at the time of sampling; disease duration and DAS28-ESR are summarized in Table 1. Seropositivity for RF and anti-CCP was 85.0% and 77.5%, respectively. Subgroup comparisons by seropositivity were not performed because of the small numbers of seronegative patients.

**Table 1.** Baseline demographic and clinical characteristics

| Characteristic | RA Patients (n=40) | Controls (n=40) | p-value |
|---|---|---|---|
| Age, years (mean ± SD) | 45.3 ± 10.2 | 44.8 ± 9.7 | 0.82 |
| Female sex, n (%) | 30 (75.0%) | 30 (75.0%) | 1.00 |
| BMI, kg/m² (mean ± SD) | 26.8 ± 3.4 | 25.9 ± 3.1 | 0.23 |
| Disease duration, years (mean ± SD) | 6.4 ± 4.1 | — | — |
| DAS28-ESR (mean ± SD) | 4.8 ± 1.3 | — | — |
| RF positive, n/N (%) | 34/40 (85.0%) | — | — |

| Characteristic | RA Patients (n=40) | Controls (n=40) | p-value |
|---|---|---|---|
| Anti-CCP positive, n/N (%) | 31/40 (77.5%) | — | — |
| csDMARD therapy, n (%) | 29 (72.5%) | — | — |
| MTX dose, mg/week (mean ± SD) | 12.5 ± 3.2 | — | — |
| NSAIDs only, n (%) | 11 (27.5%) | — | — |
| Oral corticosteroids, n (%) | 18 (45.0%) | — | — |

*DAS28-ESR: Disease Activity Score in 28 joints using ESR; all enrolled patients met the prespecified inclusion threshold of DAS28-ESR ≥ 3.2. MTX: methotrexate.*

### 3.2 Between Group Biomarker Comparisons

Shapiro–Wilk tests indicated no significant departure from normality for MDA, ESR, TNF-α, and IL-6 in either group (all $p > 0.05$; Supplementary Table S1, Panel A). GSH departed from normality in the RA group ($W = 0.932$, $p = 0.019$), and TAC departed from normality in the control group ($W = 0.931$, $p = 0.017$). CRP in the RA group did not meet the conventional criterion for non-normality ($W = 0.948$, $p = 0.065$). Independent-samples t-tests were therefore retained as the primary standardized between-group comparisons for all seven biomarkers, while Mann–Whitney U tests were used as sensitivity analyses for GSH, TAC, and CRP and as confirmatory analyses for the remaining markers (Supplementary Table S1). All seven biomarkers differed significantly between RA patients and controls (all $p < 0.001$ by both approaches). MDA, TNF-α, IL-6, CRP, and ESR were higher in RA patients, while GSH and TAC were lower. The standardized mean differences were very large (Cohen's d = 2.96–4.26), and their magnitude warrants cautious interpretation in view of the healthy-control comparison and modest sample size.

**Table 2.** Between-group biomarker comparisons (parametric results)

| Biomarker | RA Mean ± SD | Control Mean ± SD | p-value (t-test) | Cohen's d | 95% CI (Diff.) |
|---|---|---|---|---|---|
| MDA (μmol/L) | 6.47 ± 1.50 | 2.21 ± 0.61 | <0.001 | 3.72 | 3.75 to 4.77 |
| GSH (μmol/L) | 30.01 ± 8.02 | 65.10 ± 12.02 | <0.001 | −3.43 | −39.64 to −30.54 |
| TAC (mmol/L) | 0.80 ± 0.20 | 1.70 ± 0.30 | <0.001 | −3.53 | −1.01 to −0.79 |
| CRP (mg/L) | 35.03 ± 15.01 | 3.51 ± 1.50 | <0.001 | 2.96 | 26.77 to 36.27 |
| ESR (mm/hr) | 55.02 ± 20.03 | 12.01 ± 4.51 | <0.001 | 2.96 | 36.55 to 49.47 |
| TNF-α (pg/mL) | 45.01 ± 12.02 | 8.01 ± 2.50 | <0.001 | 4.26 | 33.14 to 40.86 |
| IL-6 (pg/mL) | 30.02 ± 10.01 | 4.51 ± 1.50 | <0.001 | 3.56 | 22.32 to 28.70 |

*Mann–Whitney U analyses are provided in Supplementary Table S1 as sensitivity/confirmatory analyses. Parametric mean differences, 95% confidence intervals, and Cohen's d are shown here to provide a common standardized scale across biomarkers. Negative 95% confidence intervals indicate lower mean values in RA patients relative to controls. Figure 1 summarizes the standardized between-group effect sizes and their 95% confidence intervals.*

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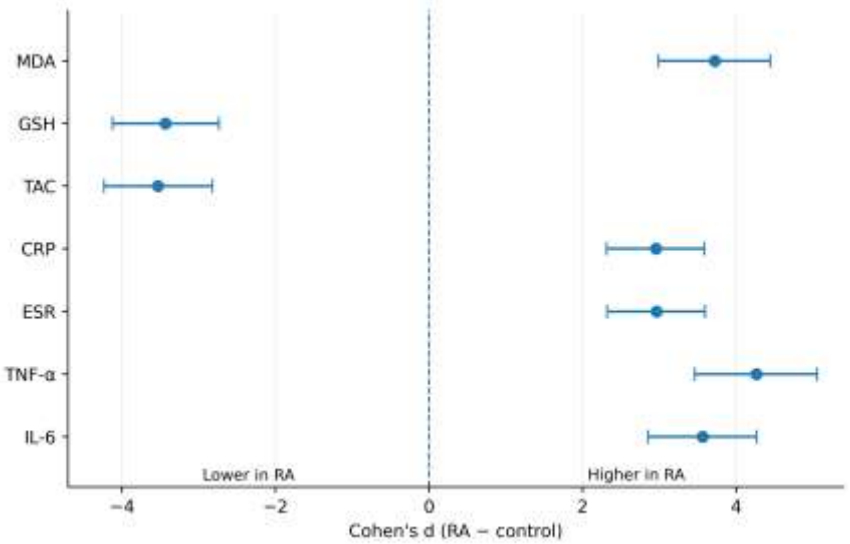


**Figure 1.** Standardized between-group differences in serum biomarkers. Points show Cohen's d for RA minus control and horizontal bars show 95% confidence intervals derived from the summary statistics in Table 2 (n = 40 per group). Positive values indicate higher levels in RA; negative values indicate lower levels in RA.

### 3.3 Exploratory Pooled Correlation Analysis

The pooled Pearson correlation matrix (Table 3, Figure 2) showed positive correlations among inflammatory markers and between MDA and inflammatory markers, together with inverse correlations between antioxidant markers and inflammatory measures. Most coefficients met the Bonferroni-adjusted significance threshold. The GSH–ESR correlation ($r = -0.30$) was nominally significant before correction ($p \approx 0.007$) but did not meet the adjusted threshold ($\alpha = 0.0024$). Because all biomarkers also showed strong case-control separation, these pooled coefficients may partly reflect group membership and should not be interpreted as evidence of within-RA biological coupling without patient-only or group-adjusted analyses.

**Table 3.** Exploratory pooled Pearson correlation matrix (r values, n = 80)

| | MDA | GSH | TAC | CRP | ESR | TNF-α | IL-6 |
|---|---|---|---|---|---|---|---|
| MDA | 1.00 | −0.40** | −0.50** | 0.60** | 0.50** | 0.70** | 0.60** |
| GSH | −0.40** | 1.00 | 0.60** | −0.40** | −0.30‡ | −0.50** | −0.40** |
| TAC | −0.50** | 0.60** | 1.00 | −0.50** | −0.40** | −0.60** | −0.50** |
| CRP | 0.60** | −0.40** | −0.50** | 1.00 | 0.70** | 0.80** | 0.70** |
| ESR | 0.50** | −0.30‡ | −0.40** | 0.70** | 1.00 | 0.60** | 0.60** |
| TNF-α | 0.70** | −0.50** | −0.60** | 0.80** | 0.60** | 1.00 | 0.80** |
| IL-6 | 0.60** | −0.40** | −0.50** | 0.70** | 0.60** | 0.80** | 1.00 |

*** $p < 0.0024$ using the Bonferroni-adjusted significance threshold ($\alpha = 0.05/21$); ‡ $p < 0.05$ before correction but not significant after correction ($r = -0.30$, $p \approx 0.007$, $df = 78$).*

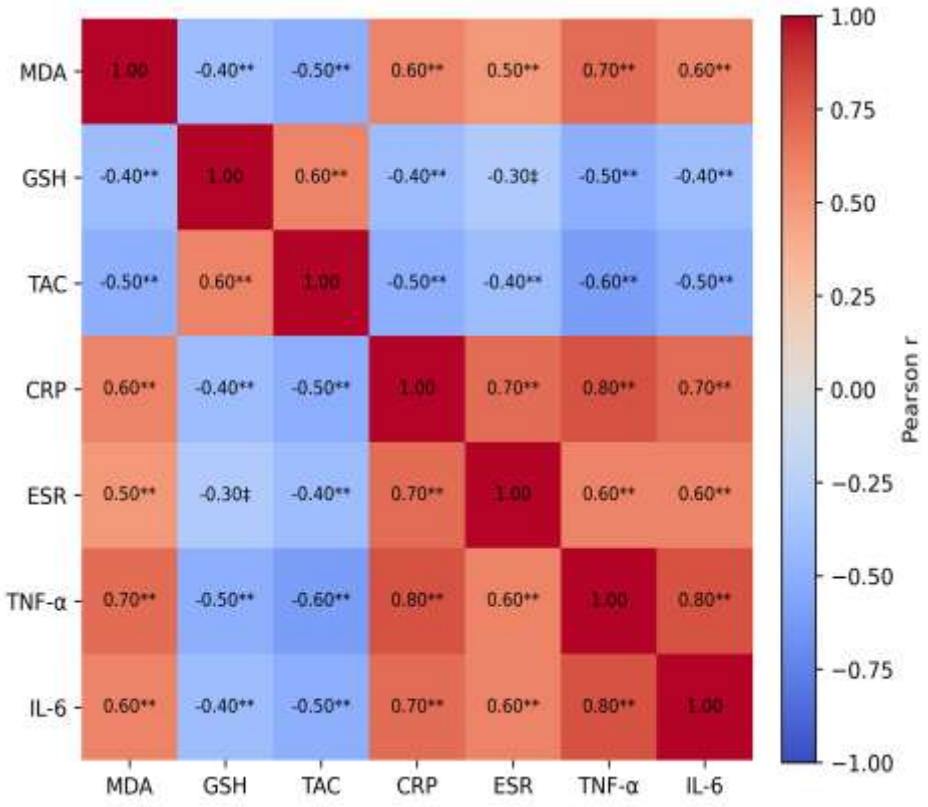


**Figure 2.** Heat map of the exploratory pooled Pearson correlation matrix across the seven biomarkers (r values from Table 3). ** $p < 0.0024$ (Bonferroni-adjusted); ‡ $p < 0.05$ before correction only (GSH–ESR). Pooled correlations may be influenced by case-control group separation.

### 3.4 Exploratory Study-Group Discrimination

Using the directional Mann–Whitney rank statistics reported in Supplementary Table S1, rank-derived AUC values were 1.000 for TNF-α, MDA, IL-6, TAC, and GSH, 0.993 for CRP, and 0.975 for ESR after orienting each marker so that higher scores indicated RA (Table 4). These values indicate near-complete separation of the established RA cases from healthy controls in this sample. They should not be interpreted as validated clinical diagnostic accuracy because the design did not include symptomatic or inflammatory disease comparator groups, and the extreme case-control separation is likely to overstate performance in routine practice. The sensitivity and specificity values in Table 4 are retained only as descriptive operating points at the reported cut-offs.

**Table 4.** Exploratory study-group discrimination by biomarker

| Biomarker | Rank-derived AUC | Empirical 95% CI | Cut-off | Sens. (%) | Spec. (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|
| TNF-α (pg/mL) | 1.000 | — | >15.2 | 97.5 | 97.5 | 97.5 | 97.5 |
| MDA (μmol/L) | 1.000 | — | >3.8 | 95.0 | 97.5 | 97.4 | 95.1 |
| IL-6 (pg/mL) | 1.000 | — | >8.5 | 95.0 | 95.0 | 95.0 | 95.0 |

| Biomarker | Rank-derived AUC | Empirical 95% CI | Cut-off | Sens. (%) | Spec. (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|
| TAC (mmol/L) | 1.000 | — | <1.25 | 92.5 | 95.0 | 94.9 | 92.7 |
| GSH (μmol/L) | 1.000 | — | <48.5 | 90.0 | 95.0 | 94.7 | 90.5 |
| CRP (mg/L) | 0.993 | — | >8.0 | 90.0 | 92.5 | 92.3 | 90.2 |
| ESR (mm/hr) | 0.975 | — | >22 | 85.0 | 87.5 | 87.2 | 85.4 |

*AUC values were recalculated from the directional Mann–Whitney statistics in Supplementary Table S1 using AUC = U/(40 × 40), with direction reversed for GSH and TAC because lower values indicated RA. Empirical AUC confidence intervals and pairwise DeLong comparisons are not reported. PPV and NPV reflect the 50% case-control sampling ratio and are not population predictive values.*

### 3.5 Exploratory Two-Predictor Logistic Model

To avoid unstable high-dimensional modeling with only 40 RA cases, inference was limited to the parsimonious two-predictor model containing MDA and TNF-α, representing oxidative and inflammatory domains. Both markers were associated with RA case status (Table 5): TNF-α OR = 1.41 (95% CI 1.18–1.68) per pg/mL and MDA OR = 3.85 (95% CI 2.11–7.02) per μmol/L. The model showed an apparent Nagelkerke $R^2$ of 0.89 and apparent classification accuracy of 98.8%. These are in-sample estimates only; no bootstrap-corrected or cross-validated performance estimate is claimed, and the model should be regarded as exploratory rather than as a validated prediction tool.

**Table 5.** Exploratory two-predictor binary logistic regression model

| Predictor | β (SE) | Wald $\chi^2$ | p-value | OR | 95% CI for OR |
|---|---|---|---|---|---|
| TNF-α (pg/mL) | 0.344 (0.090) | 14.62 | <0.001 | 1.41 | 1.18–1.68 |
| MDA (μmol/L) | 1.349 (0.307) | 19.31 | <0.001 | 3.85 | 2.11–7.02 |
| Constant | −18.74 (4.12) | 20.65 | <0.001 | — | — |

*Model summary: Nagelkerke $R^2$ = 0.89; apparent classification accuracy = 98.8%. These values are in-sample estimates and should not be interpreted as internally or externally validated predictive performance.*

## 4. DISCUSSION

### 4.1 Interpretation of Findings

The between-group results confirm the known biology of RA that oxidative stress and pro-inflammatory cytokine signaling are linked by bidirectional pathways that involve reactive oxygen species, NF-κB activation, synovial inflammation, and antioxidant defense [6,25–29,40,41]. In the current cohort, MDA and inflammatory markers were increased and GSH and TAC were decreased in RA as compared to healthy controls. The pooled correlation pattern was directionally compatible with this framework, but was not a sufficient measure to determine within-RA mechanistic coupling as the coefficient could be inflated due to a high level of separation between cases and controls. Correlation matrix-based conclusions must be made based on patient-only or group-adjusted analyses.

### 4.2 Comparison with Previous Literature

RA has been shown to have increased MDA and decreased antioxidant defense in Egyptian, Turkish, Iranian, and Iraqi populations [12,13,33,34,36]. Overall, the present pattern is quite consistent with previous evidence for redox imbalance in RA. There have also been some recent case-control studies in the Iraqi population which have reported very strong separations between RA patients and healthy controls using inflammatory cytokines [38], showing that in highly contrasted case-control samples, near-perfect separations can be achieved. In contrast, Abdullah et al. [37] investigated immune mediated inflammatory diseases, excluding RA, and did not see significant differences for some of the markers investigated but is unable to validate the present RA-focused results. Anti-CCP, TNF-α, ESR and CRP were found to be associated with the disease activity in Iraqi RA patients [39]. As a whole, the literature backs up the biological relevance of the pathways, and the importance of clinically representative comparator groups and longitudinal validation.

### 4.3 Potential Clinical Relevance and Future Research

The measurement of MDA by TBARS is technically less complex than the ELISA of cytokines, and may appeal for additional study in environments where more complex immunological assays are not available. But cost-effectiveness, longitudinal monitoring performance, treatment response and incremental value beyond established clinical measures were not assessed for the present study. Thus, current results indicate that MDA, TAC and cytokine markers are worth considering as potential markers for further validation, but not a clinically validated biomarker panel. Further research is needed to involve patients with other inflammatory and musculoskeletal diseases, to investigate the relationships of OSS with DAS28 in the RA cohort, and to assess if OSS markers are superior to traditional inflammatory parameters (CRP and ESR) to predict or monitor disease activity.

### 4.4 Limitations

There are a number of limitations to be taken into account when interpreting data. The cross sectional case-control design does not allow for causal inferences and does not provide information about the utility for longitudinal monitoring or about treatment responsiveness. Second, healthy controls were selected from hospital staff and community volunteers, instead of via the same clinical referral pathway as cases, which can lead to exaggerated biomarker differences and/or discrimination from real-world diagnostic scenarios. Third, many RA patients were receiving csDMARDs and/or corticosteroids, but the sample was not large enough for robust treatment-stratified analysis; medication-related confounding therefore remains possible. Fourth, dietary and physical-activity data, which can influence oxidative-stress markers, were not collected. Fifth, the very large standardized effects and near-complete rank separation exceed typical expectations and require replication with verified participant-level data. Sixth, the correlation analysis pooled RA patients and controls; because group membership is strongly associated with biomarker levels, pooled correlations may partly represent between-group structure rather than within-RA biological relationships. Finally, the exploratory AUC values describe separation of established RA cases from healthy controls, and the two-predictor logistic model reports in-sample performance only. Neither analysis should be interpreted as validated clinical diagnostic performance. Larger multicentre studies with clinical comparator groups, patient-only or group-adjusted correlation analyses, prespecified multivariable models, and external validation are required before clinical translation.

## 5. CONCLUSIONS

This study found marked differences in oxidative-stress, antioxidant, acute-phase, and pro-inflammatory biomarkers between Iraqi patients with RA and matched healthy controls. RA patients had higher MDA, CRP, ESR, TNF-α, and IL-6 and lower GSH and TAC, supporting the presence of concurrent redox imbalance and systemic inflammation. Exploratory pooled correlations were directionally consistent with established redox–inflammatory biology but may partly reflect case-control group separation. Likewise, the very high study-group discrimination and strong in-sample two-marker model should be interpreted cautiously because the comparison involved established RA cases and healthy controls and lacked external validation. These findings identify MDA, antioxidant measures, and inflammatory cytokines as candidates for further study, but they do not establish a diagnostic or monitoring panel. Validation in larger, clinically representative and longitudinal Iraqi cohorts is required before clinical application.

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**Author Contributions**

Conceptualization: Noor Ali Salman, Marwa Ali Hadi. Methodology: Noor Ali Salman, Marwa Ali Hadi. Investigation: Marwa Ali Hadi, Shaima Basil Salman. Formal analysis: Noor Ali Salman. Data curation: Noor Ali Salman, Shaima Basil Salman. Resources: Marwa Ali Hadi. Writing – original draft: Noor Ali Salman. Writing – review and editing: Noor Ali Salman, Marwa Ali Hadi, Shaima Basil Salman. Visualization: Noor Ali Salman. Supervision: Marwa Ali Hadi. Project administration: Noor Ali Salman. All authors have read, reviewed, and approved the final manuscript, in accordance with the CRediT taxonomy.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Declaration of Generative AI**

Generative artificial intelligence was used for editorial support and statistical-consistency checking during manuscript revision. The authors reviewed all suggested changes, verified the scientific content against the study records, and retain full responsibility for the final manuscript, data, analyses, and interpretation.

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**Supplementary Material**

**Supplementary Table S1.** Shapiro–Wilk Normality Tests and Mann–Whitney U Sensitivity Analyses

**Panel A: Shapiro–Wilk Normality Tests (n = 40 per group)**

| Biomarker | Unit | Group | W-statistic | p-value | Normality conclusion |
|---|---|---|---|---|---|
| MDA | μmol/L | RA | 0.960 | 0.163 | Normal (p > 0.05) |
| MDA | μmol/L | Control | 0.979 | 0.650 | Normal (p > 0.05) |
| GSH | μmol/L | RA | 0.932 | 0.019 | **Non-normal (p < 0.05)** |
| GSH | μmol/L | Control | 0.977 | 0.565 | Normal (p > 0.05) |
| TAC | mmol/L | RA | 0.956 | 0.118 | Normal (p > 0.05) |
| TAC | mmol/L | Control | 0.931 | 0.017 | **Non-normal (p < 0.05)** |
| CRP | mg/L | RA | 0.948 | 0.065 | No significant departure (p > 0.05) |
| CRP | mg/L | Control | 0.985 | 0.862 | Normal (p > 0.05) |
| ESR | mm/hr | RA | 0.979 | 0.647 | Normal (p > 0.05) |
| ESR | mm/hr | Control | 0.991 | 0.986 | Normal (p > 0.05) |
| TNF-α | pg/mL | RA | 0.949 | 0.069 | Normal (p > 0.05) |
| TNF-α | pg/mL | Control | 0.991 | 0.981 | Normal (p > 0.05) |
| IL-6 | pg/mL | RA | 0.971 | 0.398 | Normal (p > 0.05) |
| IL-6 | pg/mL | Control | 0.985 | 0.852 | Normal (p > 0.05) |

*Bold values indicate significant departure from normality ($p < 0.05$). Independent-samples t-tests were retained as the primary standardized between-group comparisons, while Mann–Whitney U tests were used as sensitivity analyses for GSH, TAC, and CRP and as confirmatory analyses for MDA, ESR, TNF-a, and IL-6.*

**Panel B: Mann–Whitney U Sensitivity Analyses for GSH, TAC, and CRP**

Mann–Whitney U tests were applied as sensitivity analyses for GSH, TAC, and CRP. The directional U statistic is reported with RA as group 1.

| Biomarker | Unit | RA (Median, IQR) | Control (Median, IQR) | Directional U (RA = group 1) | p-value |
|---|---|---|---|---|---|
| GSH | μmol/L | 29.85 (24.10–36.12) | 65.02 (57.40–72.80) | 0.0 | <0.001 |
| TAC | mmol/L | 0.80 (0.65–0.95) | 1.70 (1.49–1.91) | 0.0 | <0.001 |
| CRP | mg/L | 38.07 (25.40–44.58) | 3.54 (2.61–4.35) | 1,589.0 | <0.001 |

*All three sensitivity analyses yielded $p < 0.001$, consistent with the primary parametric comparisons. Directional U values are reported with RA as group 1; for markers lower in RA, the direction is reversed when deriving an AUC.*

**Panel C: Mann–Whitney U Confirmatory Analyses for the Remaining Biomarkers**

For completeness, Mann–Whitney U tests were also applied to MDA, ESR, TNF-α, and IL-6 as confirmatory analyses.

| Biomarker | RA (Median, IQR) | Control (Median, IQR) | Directional U (RA = group 1) | p-value |
|---|---|---|---|---|
| MDA | 6.52 (5.48–7.41) | 2.18 (1.82–2.59) | 1,600.0 | <0.001 |
| ESR | 56.64 (39.77–71.07) | 12.21 (8.90–14.91) | 1,560.0 | <0.001 |
| TNF-α | 45.10 (36.20–54.30) | 7.85 (6.10–9.80) | 1,600.0 | <0.001 |
| IL-6 | 30.05 (22.80–37.40) | 4.48 (3.52–5.45) | 1,600.0 | <0.001 |

*All four confirmatory Mann–Whitney U analyses yielded $p < 0.001$, consistent with the parametric results in Table 2. Directional U values are reported with RA as group 1.*