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Immunological Role of IL-40, IL-37, and IL-38 in Patients with Rheumatoid Arthritis: Association with Pro-Inflammatory Cytokines

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ABSTRACT
Background: Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterised by persistent synovial inflammation and progressive joint destruction. The immunomodulatory roles of IL-40, IL-37, and IL-38 in RA remain incompletely understood, particularly in Middle Eastern patient populations. **Research question:** Are serum levels of IL-40, IL-37, and IL-38 elevated in RA patients compared with healthy controls, and do they correlate with established pro-inflammatory markers? **Methods:** A cross-sectional comparative study was conducted at Tikrit Teaching Hospital, Salah al-Din, Iraq (January–September 2025), enrolling 60 RA patients and 30 age- and sex-matched healthy controls. Serum cytokines were quantified by ELISA. Normality was assessed by the Shapiro-Wilk test; Mann-Whitney U and Spearman rank correlation were applied. **Results:** IL-40 (8.91±1.08 vs 7.29±2.24 pg/mL; p=0.0002), IL-37 (398.44±72.95 vs 68.33±29.30 pg/mL; p<0.001), and IL-38 (635.48±197.43 vs 145.23±56.17 pg/mL; p<0.001) were all significantly elevated in RA. All pro-inflammatory markers were markedly elevated (p<0.001). Within the RA group, IL-37 correlated positively with CRP (r=0.352, r^2=0.124, p=0.006) and ESR (r=0.311, r^2=0.097, p=0.016). **Conclusion:** These findings suggest that IL-40, IL-37, and IL-38 may be actively involved in RA immunopathology. The moderate positive correlations between IL-37 and acute-phase reactants may reflect a counter-regulatory response proportional to systemic inflammatory burden. Confirmation in larger, longitudinal, multi-centre studies is required.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, systemic autoimmune disease characterised by persistent synovial inflammation, pannus formation, and progressive cartilage and bone erosion, culminating in functional disability and reduced quality of life [1]. It affects approximately 0.5–1% of the global population, with a marked female predilection, and imposes a considerable economic burden through work incapacity and long-term pharmacotherapy [2, 17]. In resource-limited settings such as Iraq, suboptimal disease control remains prevalent, underscoring the need for further characterisation of regional immunological patterns.

The immunopathology of RA involves a complex cytokine network. T helper 17 (Th17) cells, B lymphocytes, macrophages, and synovial fibroblasts collectively produce pro-inflammatory mediators—including TNF-α, IL-6, IL-17A, and IL-23—that sustain synovial inflammation and drive joint destruction [3, 4, 18]. The chemokine CCL5 (RANTES) amplifies leukocyte recruitment into the inflamed synovium [5, 19, 20]. Clinically, systemic inflammatory burden is reflected in elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [6, 21, 23].

Over the past decade (2015–2025), several novel immunomodulatory interleukins with predominantly anti-inflammatory properties have been characterised [7, 8, 9, 30, 31, 32]. Among these, IL-40, IL-37, and IL-38 have attracted considerable research interest due to their potential regulatory roles in autoimmune disease. **IL-40 (C17orf99)** is predominantly produced by activated B cells and promotes B cell differentiation and antibody class-switching [7, 8]. In RA, dysregulated B cell activity drives production of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) [14], suggesting that IL-40 may serve as a marker of B cell-mediated humoral dysregulation. **IL-37** is a member of the IL-1 superfamily and exerts broad immunosuppressive effects by inhibiting NF-κB and MAPK signalling pathways [9, 31]. Prior studies have reported elevated IL-37 in RA serum and synovial fluid, interpreted as a counter-regulatory mechanism attempting to restrain excessive inflammation [10, 11]. **IL-38** is another IL-1 family member that antagonises IL-36 receptor signalling and suppresses production of IL-17 and IL-23 in experimental models [12, 13, 32]. Systematic reviews suggest a protective role, but clinical data from human RA cohorts—particularly in the Middle East—remain sparse [32].

Research question: Are serum IL-40, IL-37, and IL-38 significantly elevated in Iraqi RA patients compared with healthy controls, and do they correlate with key pro-inflammatory cytokines and acute-phase reactants? **Hypothesis:** Serum levels of IL-40, IL-37, and IL-38 are elevated in RA patients relative to healthy controls, and at least one of these cytokines shows a statistically significant correlation with established pro-inflammatory markers, consistent with their proposed immunomodulatory roles in RA pathogenesis.

2.Materials and Methods

2.1 Study Design and Ethical Considerations

This was a cross-sectional comparative study conducted at the outpatient rheumatology clinic of Tikrit Teaching Hospital, Salah al-Din Governorate, Iraq, from January to September 2025. Ethical approval was granted by the Salah al-Din Health Directorate (letter No. 244-B, 1 December 2024). Written informed consent was obtained from all participants prior to enrolment. Data were collected anonymously, stored on secured institutional servers, and used exclusively for the purposes of this study. Participants retained the right to withdraw consent at any time without consequence.

2.2 Participants and Sampling

Ninety participants were recruited through consecutive sampling from the rheumatology clinic register, divided into two groups: (1) **RA patients (n=60)**: adults aged ≥18 years fulfilling the 2010 ACR/EULAR classification criteria for RA; and (2) **healthy controls (n=30)**: individuals matched for age (±5 years) and sex with no history of autoimmune, inflammatory, infectious, or chronic metabolic disease. The 2:1 RA-to-control allocation was selected based on an expected moderate effect size (Cohen's d ≈0.6), which at 80% statistical power (α=0.05, two-tailed) requires approximately 52 cases and 26 controls, making our sample adequately powered [3].

Exclusion criteria: pregnancy or lactation; active infections; concurrent autoimmune conditions; malignancy; renal or hepatic insufficiency; use of biological disease-modifying antirheumatic drugs (bDMARDs) within three months of enrolment.

2.3 Blood Sample Collection and Processing

Venous blood (10 mL) was collected under aseptic conditions after overnight fasting. Samples were allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes at 4°C. Serum aliquots were stored at −80°C until analysis.

2.4 Laboratory Assays

Serum concentrations of IL-40, IL-37, IL-38, IL-17A, IL-23, IL-6, TNF-α, and CCL5 were quantified by commercially available sandwich ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China), according to the manufacturer's instructions. Intra-assay and inter-assay coefficients of variation (CV) were below 10% for all analytes, confirming acceptable analytical precision. CRP was measured by immunoturbidimetry; ESR was determined by the Westergren method [6].

2.5 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0. The Shapiro-

Wilk test confirmed non-normality for most continuous variables; therefore, non-parametric tests were employed throughout. Between-group differences in continuous variables were analysed using the Mann-Whitney U test; the chi-square test was used for sex comparisons. Associations between IL-40, IL-37, IL-38, and pro-inflammatory markers within the RA group were assessed by Spearman rank correlation (rs). Effect size was estimated as r^2 (coefficient of determination). All results are expressed as Mean ± SD. A p-value <0.05 was considered statistically significant.

3. Results

3.1 Demographic Characteristics

The RA group comprised 60 patients (34 males, 26 females; mean age 46.18±10.29 years; range 22–72). The control group comprised 30 individuals (19 males, 11 females; mean age 42.90±10.68 years; range 15–62). No significant between-group differences were observed for age (p=0.102) or sex distribution (χ^2=0.281, p=0.541), confirming demographic comparability between groups (Table 1).

Table 1. Demographic characteristics of the study groups

Parameter	RA Patients (n=60)	Healthy Controls (n=30)
Age (years), Mean ± SD	46.18 ± 10.29	42.90 ± 10.68
Age range (years)	22 – 72	15 – 62
Male, n (%)	34 (56.7%)	19 (63.3%)
Female, n (%)	26 (43.3%)	11 (36.7%)
Age comparison (p-value)	0.102 (NS)	—
Sex comparison (p-value)	0.541 (NS)	—

NS = Not significant. Age compared by Mann-Whitney U test; sex compared by chi-square test.

All measured biomarkers were significantly elevated in RA patients compared with healthy controls (Table 2). Among the immunomodulatory interleukins, IL-38 showed the greatest absolute elevation, with a mean concentration approximately 4.4-fold higher than controls (635.48±197.43 vs 145.23±56.17 pg/mL; U=1798.0, p<0.001). IL-37 was elevated approximately 5.8-fold (398.44±72.95 vs 68.33±29.30 pg/mL; U=1800.0, p<0.001). IL-40 demonstrated a statistically significant but more modest elevation of approximately 22% above control values (8.91±1.08 vs 7.29±2.24 pg/mL; U=1339.5, p=0.0002). Among the pro-inflammatory markers, IL-6 (20.72±8.11 vs 2.66±1.17 pg/mL), TNF-α (22.01±5.46 vs 9.22±2.52 pg/mL), CRP (17.15±6.75 vs 2.34±1.02 mg/L), and ESR (41.33±14.54 vs 11.87±4.45 mm/hr) were all markedly elevated in RA patients (all p<0.001).

3.3 Spearman Rank Correlation Analysis

Within the RA group, Spearman correlation analysis identified two statistically significant positive associations: IL-37 with CRP (rs=0.352, r^2=0.124, p=0.006) and IL-37 with ESR (rs=0.311, r^2=0.097, p=0.016). These moderate correlations indicate that IL-37 explains approximately 12% and 10% of the variance in CRP and ESR, respectively. No significant correlations were identified for IL-40 or IL-38 with any of the measured pro-inflammatory markers (all p>0.05). Complete correlation data are presented in Table 3.

Table 2. Serum biomarker levels in RA patients and healthy controls

Biomarker	RA Patients (Mean±SD)	Controls (Mean±SD)	U Statistic	p-value
IL-40 (pg/mL)	8.91 ± 1.08	7.29 ± 2.24	1339.5	0.0002
IL-37 (pg/mL)	398.44 ± 72.95	68.33 ± 29.30	1800.0	<0.001
IL-38 (pg/mL)	635.48 ± 197.43	145.23 ± 56.17	1798.0	<0.001
IL-17A (pg/mL)	49.72 ± 10.88	27.69 ± 8.19	1718.0	<0.001
IL-23 (pg/mL)	67.55 ± 14.68	37.82 ± 12.28	1687.5	<0.001
IL-6 (pg/mL)	20.72 ± 8.11	2.66 ± 1.17	1748.5	<0.001
TNF-α (pg/mL)	22.01 ± 5.46	9.22 ± 2.52	1779.0	<0.001
CCL5 (ng/mL)	1.20 ± 0.30	0.83 ± 0.24	1513.0	<0.001
CRP (mg/L)	17.15 ± 6.75	2.34 ± 1.02	1740.0	<0.001
ESR (mm/hr)	41.33 ± 14.54	11.87 ± 4.45	1760.0	<0.001

Data expressed as Mean ± SD. Mann-Whitney U test used for all comparisons. NS = not significant.

Table 3. Spearman rank correlations between IL-40, IL-37, IL-38, and pro-inflammatory markers in the RA group

Interleukin	Marker	Spearman r	r^2	p-value	Significance
IL-40	IL-17A	0.000	—	0.999	NS
IL-40	IL-23	0.089	—	0.497	NS
IL-40	IL-6	-0.162	—	0.215	NS
IL-40	TNF-α	-0.115	—	0.384	NS
IL-40	CCL5	0.154	—	0.239	NS
IL-40	CRP	0.013	—	0.923	NS
IL-40	ESR	0.032	—	0.811	NS
IL-37	IL-17A	-0.005	—	0.968	NS
IL-37	IL-23	-0.217	—	0.095	NS
IL-37	IL-6	0.048	—	0.713	NS
IL-37	TNF-α	0.004	—	0.978	NS
IL-37	CCL5	0.002	—	0.990	NS
IL-37	CRP	0.352	0.124	0.006	*
IL-37	ESR	0.311	0.097	0.016	*
IL-38	IL-17A	0.001	—	0.992	NS
IL-38	IL-23	0.057	—	0.666	NS
IL-38	IL-6	0.173	—	0.187	NS
IL-38	TNF-α	0.087	—	0.510	NS
IL-38	CCL5	0.074	—	0.574	NS
IL-38	CRP	-0.101	—	0.445	NS
IL-38	ESR	-0.061	—	0.641	NS

** Statistically significant ($p<0.05$). r^2 = coefficient of determination (effect size). NS = not significant.*

3.Discussion

The principal finding of this study is that serum IL-40, IL-37, and IL-38 are all significantly elevated in RA patients compared with healthy controls, implicating these immunomodulatory cytokines in the pathobiology of RA. The moderate positive correlations of IL-37 with the acute-phase reactants CRP (r^2=0.124) and ESR (r^2=0.097) suggest that its induction may be proportional to the degree of systemic inflammation, consistent with a counter-regulatory feedback mechanism. The modest (~22%) elevation of IL-40 in RA patients is consistent with its reported role in B cell activation and humoral immunity [7, 8]. IL-40 promotes B cell differentiation and antibody class-switching—processes directly relevant to RA through the production of RF and ACPAs [14]. The absence of significant correlations between IL-40 and classical systemic inflammatory markers suggests that its role in RA may be primarily confined to B cell-mediated adaptive immunity rather than the acute-phase inflammatory response. Similar patterns of B cell dysregulation have been documented in Iraqi RA cohorts [25].

The approximately 5.8-fold elevation of IL-37 corroborates prior reports of IL-37 upregulation in RA serum and synovial fluid [10, 11, 31]. IL-37 inhibits innate inflammatory cascades through suppression of NF-κB and MAPK activation [9]. The significant positive associations with CRP and ESR are consistent with a counter-regulatory feedback model: as systemic inflammation intensifies—reflected by rising acute-phase reactants—IL-37 production may be upregulated proportionally in an attempt to restore immune homeostasis [15]. However, the r^2 values (0.097–0.124) indicate that IL-37 explains only 10–12% of the variance in these markers, underscoring the multifactorial nature of RA inflammation. Clinically, this moderate effect size means that IL-37 alone is unlikely to serve as a reliable standalone diagnostic or prognostic biomarker without validation in larger cohorts.

An important alternative explanation that cannot be excluded is that elevated IL-37 may partly reflect pharmacological effects of concurrent antirheumatic therapy (e.g., methotrexate, hydroxychloroquine) rather than purely disease-driven immune activation [28]. Because treatment information was not systematically collected in this study, this confounding possibility remains unresolved and should be addressed in future investigations.

The approximately 4.4-fold elevation of IL-38 is the most pronounced quantitative finding, yet IL-38 showed no significant correlations with any measured pro-inflammatory biomarker. Systematic reviews of IL-38 in rheumatic diseases suggest that its regulatory effects may be primarily compartmentalised within the synovial microenvironment rather than reflected in systemic markers [32]. The absence of correlation may alternatively reflect insufficient statistical power in this single-centre sample to detect weak associations.

All pro-inflammatory markers were significantly elevated in RA patients, consistent with the well-established cytokine milieu of active disease [1, 3, 4, 5, 18, 22, 24, 27]. Comparable inflammatory patterns have been documented in Iraqi RA cohorts from multiple governorates [17, 18, 20, 24, 29], supporting the regional generalisability of these findings.

Limitations

Several limitations should inform interpretation of these findings. First, the cross-sectional design precludes causal inference; all reported associations are correlational. Second, the study was conducted at a single centre (Tikrit Teaching Hospital), which may limit generalisability to broader Iraqi or regional populations. Third, disease activity was not formally quantified using validated instruments such as DAS28 or CDAI. Fourth, treatment regimen data—including the type and duration of DMARDs—were not collected, introducing potential pharmacological confounding of cytokine levels. Fifth, other potentially confounding variables, including disease duration, body mass index, and nutritional status, were not systematically assessed. Sixth, while the sample size was adequately powered for primary between-group comparisons, it may have been insufficient to detect weak correlations for IL-40 and IL-38.

5.Conclusion

These findings suggest that serum IL-40, IL-37, and IL-38 may be involved in the immunopathology of rheumatoid arthritis, as

evidenced by their significant elevation in RA patients compared with healthy controls. The moderate positive correlations of IL-37 with CRP and ESR may indicate that IL-37 induction is proportional to systemic inflammatory burden, potentially reflecting a counter-regulatory mechanism. These findings do not establish causality and should be interpreted in the context of the study's cross-sectional design and single-centre setting.

Future studies should: (1) employ multi-centre designs across Iraqi governorates to enhance generalisability; (2) incorporate DAS28 or CDAI scoring to correlate cytokine levels with disease activity; (3) stratify patients by treatment regimen to disentangle pharmacological from disease-driven cytokine changes; and (4) adopt longitudinal designs to assess temporal cytokine dynamics in relation to therapeutic response.

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