https://doi.org/10.15218/zjms.2026.038

Macrophage polarization induced immune dysregulation in rheumatoid arthritis

Received: 21/08/2025 Accepted: 19/10/2025

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Abstract

Background and objective: Rheumatoid arthritis (RA) is one of the most prominent inflammatory autoimmune illnesses, causing polyarticular synovitis. The precise causes of RA are still unknown; however, chronic inflammation patterns are influenced by a combination of immunological, environmental, and epigenetic factors. Anti-CCP antibodies are a particular biomarker for RA diagnosis and severity. Alterations of complement components and pro- and anti-inflammatory cytokines are critical to the pathogenesis of RA. The current study intended to compare and examine the immunological properties of anti-CCP, IL-10, IL-17, and C5a antibodies in samples from RA patients and healthy individuals. The study evaluated medication exposure and various immune markers simultaneously to enhance immunological profiling, clinical accuracy, and classification of patients with RA

Methods: The Cobas e 411 analyzer was used to measure the anti-CCP antibody levels in the serum of 60 RA patients and 40 healthy individuals. Enzyme-linked immunosorbent assay (ELISA) technique from the BioTek ELx800 was used to estimate the levels of IL-17, IL-10, and C5a in the sera of participants. Both groups were matched in age and ethnicity. An unpaired T-test was used to analyze data statistically using the GraphPad Prism 8 program. The ANOVA test was used for more than 2 groups.

Results: The current study indicated that patients with rheumatoid arthritis (RA) had a significantly lower concentration of IL-10 serum levels as compared to the healthy controls, yet with increased levels of IL-17, C5a, and anti-CCP antibodies. Some of the causes of the decrease in IL-10 might be chronic inflammation and the suppressive action of the pro-inflammatory cytokines on its production and signaling. Further, RA patients under corticosteroid treatment had reduced levels of IL-17, C5a, and anti-CCP antibodies compared to untreated patients, showing corticosteroid therapy lowers the inflammatory mediators without restoring the IL-10 levels.

Conclusion: It can be concluded that macrophages from rheumatoid arthritis patients shifted from anti-inflammatory (like IL-10) to pro-inflammatory (like IL-17) properties. Patients with rheumatoid arthritis had lower levels of anti-inflammatory cytokine markers like IL-10 and greater levels of pro-inflammatory cytokines like IL-17, inflammatory markers like C5a, and rheumatoid arthritis markers like anti-CCP.

Keywords: Macrophage polarization, Rheumatoid arthritis, Autoantibody.

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https://doi.org/10.15218/zjms.2026.038

Introduction

Rheumatoid arthritis is a chronic, systemic, inflammatory, autoimmune disease that affects both small and large joints but primarily infects small joints and then progresses to large joints and internal organs (1). Although it can affect individuals of any age, sex, or race, it is more prevalent among older people, and it happens two to three times more frequently in women compared to men (2). RA is also affecting about 0.5-1 percent of the global population, although the figure has changed considerably (3). RA pathogenesis is characterized by the accumulation of immune cells in the synovial tissue and a complex network of cytokines and inflammatory mediators that leads to chronic synovitis and destruction of joints (4). some of pro-inflammatory cytokines, including TNF-alpha, IL-1, IL-17, IL-6, and IL-12, contribute to joint damage, pannus, and other extra-rheumatic symptoms. These cytokines also promote the differentiation and activation of osteoclasts, leading to erosion of the bones. Also, the abnormal immune reaction that is typical of RA also involves T cells and macrophages, with their regulating molecules, such as CTLA-4 (5). IL-17, primarily produced by Th17, induces the mobilization of neutrophils and the expression of other pro-inflammatory cytokines and matrix-degrading enzymes, leading to loss of the cartilage and bone (6). Conversely, regulatory T cells and monocytes produce an anti-inflammatory cytokine called IL-10, which is used to inhibit immune response and protect organs against over inflammation. The level of IL-17/IL-10 is decisive in the regulation of immune responses and maintenance of tissue homeostasis in RA. The other notable immune system component that is implicated in the pathophysiology of RA is the complement system. Complement component C5a, one of its mediators, has the ability to act as a chemoattractant and aids in the recruitment of neutrophils and macrophages to inflammatory joints (7). C5a also promotes cytokine release and increases endothelial barrier permeability, hence increasing inflammation (8). The use of autoantibodies is also useful in the diagnosis and treatment of RA. The specificity (90-99 percent) and sensitivity (66-88 percent) of anti-cyclic citrullinated peptide (anti-CCP) antibodies are very high. The detection of these biomarkers is extremely characteristic of RA (9). These antibodies are against citrullinated proteins formed by peptidyl-arginine deiminase (PAD) enzymes in the inflamed synovial tissue, including modified fibrins, filaggrin, and vimentin (10). Anti-CCP antibodies are potentially present a decade prior to clinical symptoms and are related to worse disease and erosion of joints. Their

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pathogenicity is linked with their ability to activate the complement system (11). RA pathogenesis is also observed to be influenced by macrophages and neutrophils present in the synovial fluid. Macrophages are intense providers of the pro-inflammatory cytokines like TNF-a, IL-1, and IL-6 and are associated with the destruction of the joint as well as response to the therapy (12). Neutrophils which are abundant in the synovial fluid enhance the inflammatory process by the secretion of cytokines, activation of complement system, and endocytosis (13). Drugs such as methotrexate and anti-TNF agents indirectly modulate the pro-inflammatory cytokines that are produced by macrophages. The IL-17 and IL-10 levels may also be affected by these treatments (14). Nonetheless, owing to the inconsistent results immune biomarkers are worth using to diagnose and as part of the personalized treatment (15). Along these lines, a regional study among rheumatoid arthritis patients in Sulaymaniyah similarly explored hematological inflammatory markers, such as the neutrophil-to-lymphocyte ratio and red cell distribution width, in relation to disease activity (35).

This study comparedIL-10, IL-17, C5a, and anti-CCP in RA and healthy samples to monitor macrophage polarization and exposure to treatment to enhance immunological characterization and patient stratification among RA patients.

Experimental Procedure

Study Design

This case-control study evaluated the serum IL-10, IL-17, C5a and anti-CCP antibodies to measure the immune dysregulations in rheumatoid arthritis. The recruitment of patients was conducted in Rizgary Teaching Hospital (Erbil) and Rheumatology Department of the Surgical Teaching Hospital (Sulaymaniyah) on November 15, 2024, to January 10, 2025.

Sixty RA patients (9 men, 51 females; 18-75 years) that fit the 2010 ACR/EULAR criteria were selected and 40 healthy controls (9 males, 31 females; age- and ethnicity-matched). Pregnancy, age less than 18 and more than 75, malignancy, malnutrition as well as other autoimmune or chronic inflammatory diseases were excluded. All participants were taken through informed consent. An outline of the flow of participants, selection criteria and the variables measured are shown in Figure 1.

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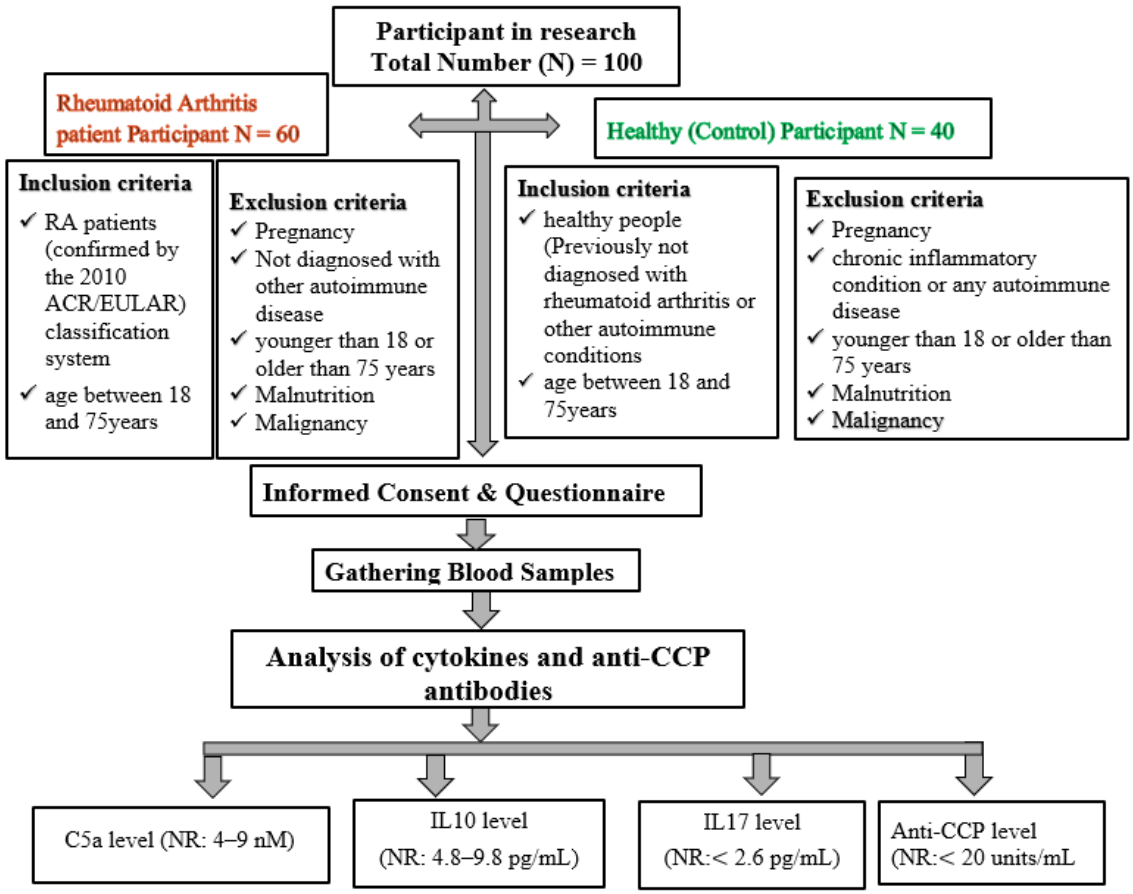

Figure 1. Participant Assessments and Design of the Study

Revealed that flowchart of the groups that was taken in the study, inclusion/exclusion criteria, evaluation of immune markers (C5a, IL-10, IL-17, anti-CCP), as well as reference ranges were identified.

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Procedures for Collecting and Managing Samples Used for Immunological Studies

Aseptic blood samples (3.5 mL) were taken from every participant, left to clot at room temperature, and centrifuged at 1000xg for 20 minutes. The aliquoted separated serum (1.5 - 2 mL) was stored either at -24°C or -80°C until analysis. The entire procedure is clearly shown in Figure 2.

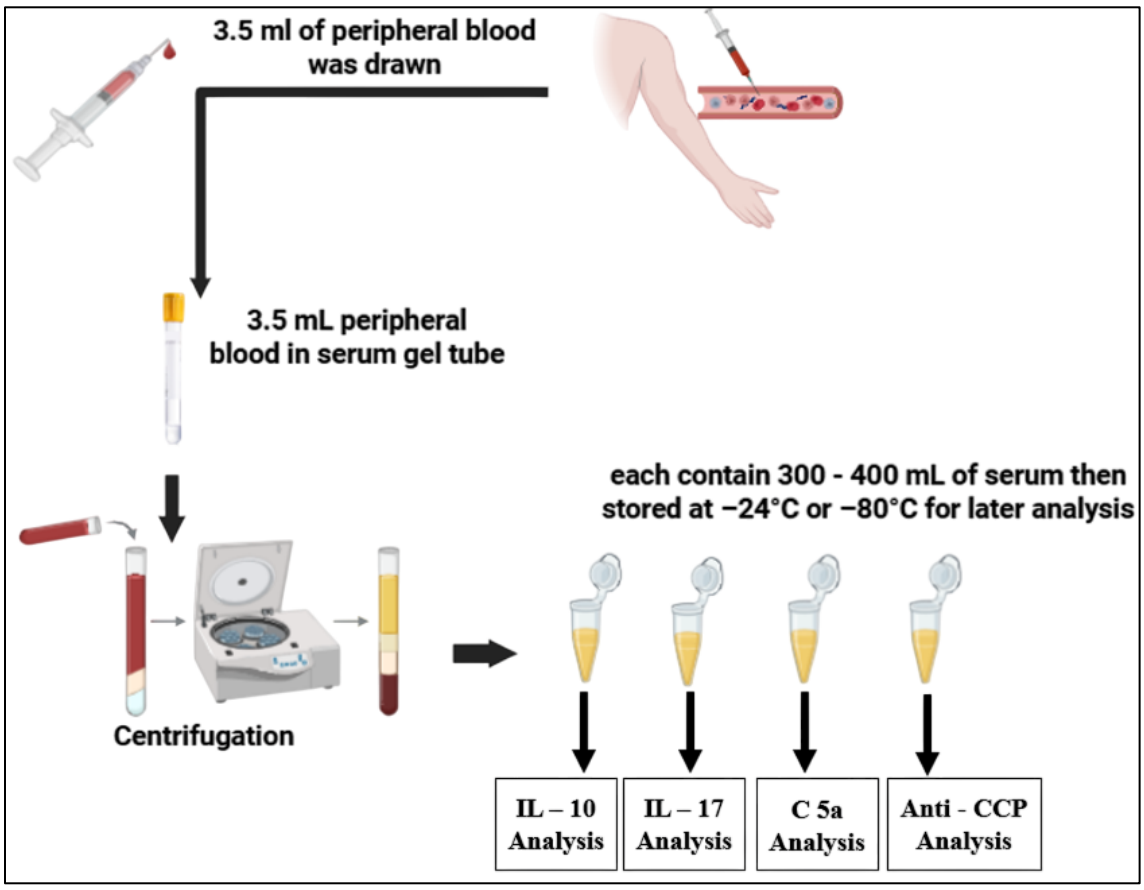

Figure 2. Blood collection, serum preparation and immunological tests

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Materials and Instruments

IL-10 (ELK1142), IL-17 (ELK2610), and C5a (ELK1051) assays were performed using commercial ELISA kits (ELK Biotech, China) and antibody detection was done using an automated Elecsys anti-CCP test (Roche Diagnostics, Germany; Cobas e 411 analyzer). The processing of samples used the standard laboratory materials, such as centrifuge, freezer, thermo-incubator, BioTek ELX800 Microplate Reader, and Cobas e 411 analyzers.

Analysis of Biomarkers and Immunological Testing

ELISA procedures were all according to manufacturer guidelines. Standards and serum samples were incubated, washed, and reacted with HRP streptavidin and TMB substrate, and absorbance was measured at 450 nm. The Cobas e 411 system was used to analyze the levels of the anti-CCP automatically. Each of the ELISA Assay Procedures is shown Figure 3.

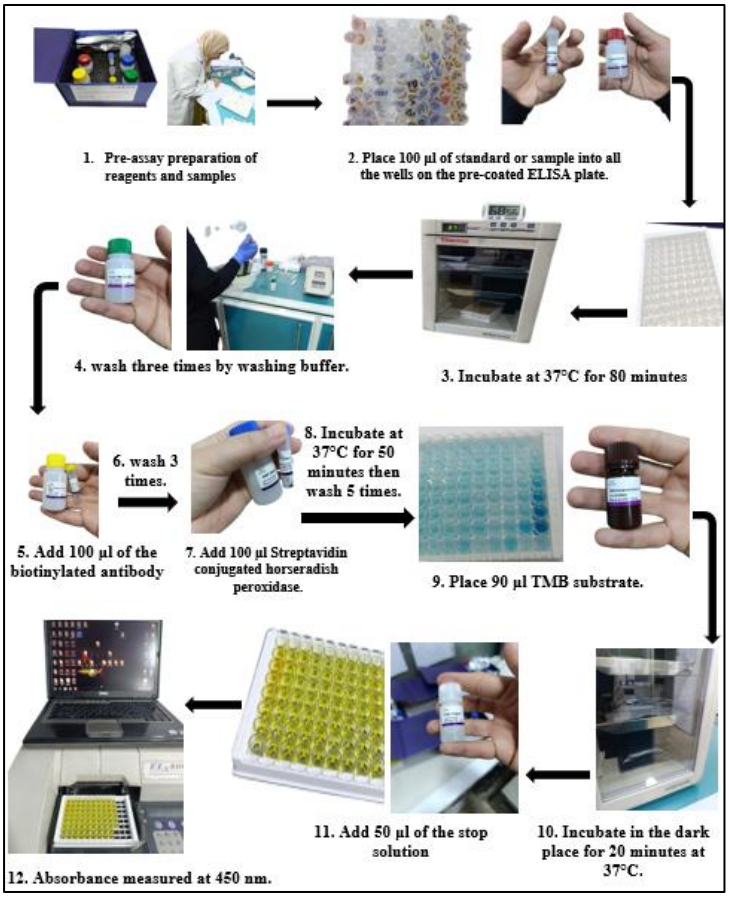

Figure 3. ELISA assay procedure overview

Procedure of ELISA implemented in the work step-by-step. During the real experimental work, photos were made to demonstrate the main stages of the process, such as reagent preparation, addition of a sample, their incubation, washing and reacting with a substrate and measuring.

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Statistical analysis

GraphPad Prism 8.2 was used to analyze data. The outcomes were presented as the mean ± SEM. One-way ANOVA was followed by the Tukey test to compare groups, and P <0.05 was taken as statistically significant.

Results

The patients were diagnosed by medical doctors based on signs, symptoms, and immune-inflammatory markers. The other forty people were controlled people who didn't have rheumatoid arthritis or any other autoimmune or inflammatory disease. Three groups were created from the data: treatment groups, age-stratified groups, and general groups.

Macrophage polarization in Rheumatoid arthritis patients

Macrophage polarization to anti-inflammatory property

Regardless of age, our results indicated that RA patients tended to have not significantly lower IL-10 levels than controls (P-value = 0.075) (Figure 4 A). Descriptive statistics of serum IL- 10 levels were shown that mean of IL-10 was lower in rheumatoid arthritis (57.19 ± 3.797) compared to control group (70.87 ± 7.563)(Table 1). An important finding was that RA patients aged 18–39 years had non-significantly lower levels of IL-10 compared to control samples of the same age range (P-value = 0.9870). In contrast, a significant difference was observed between RA patients aged 18–39 years and those aged 40–75 years (P-value = 0.0001). Additionally, there was a tendency for RA patients aged 40 to 75 to have not significantly lower levels of IL-10 compared with healthy individuals in the same age range (P-value = 0.302) (Figure 4 B). Descriptive statistics of serum IL- 10 levels were showed that mean of IL-10 was lower in RA patients (71.77 ± 3.973) aged 40 to 75 to compared to control group (84.61 ± 7.257)(Table 2). In order to examine the impact of treatment on IL-10 levels, it was shown that patients receiving bDMARDs tended to have not significantly greater IL-10 levels than those receiving other treatments (P-value = 0.870) (Figure 4, C). Descriptive statistics of serum IL- 10 levels were shown that mean of patients (128.8 ± 28.41) who received bDMARDs was more compared to other RA treatments (Table 1).

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Table 1. Descriptive statistics of serum IL- 10 levels

IL-10 (pg/ml)	Mean ± S.E. Mean	P-value
Control	70.87 ± 7.563	0.075
Patients	57.19 ± 3.797	
Control (18 - 39)	22.10 ± 2.273	0.9870
Patients (18 - 39)	1 7.34 ± 1.437	
Control (40 - 75)	84.61 ± 7.257	0.302
Patients (40 - 75)	71.77 ± 3.973	
Patients (18 - 39)	1 7.34 ± 1.437	0.0001
Patients (40 - 75)	71.77 ± 3.973	
NSAIDs	74.76 ± 24.08	0.995
sDMARDs	57.52 ±4.999	
bDMARDs	128.8 ± 28.41	0.870
Corticosteroids	47.30 ± 5.700	

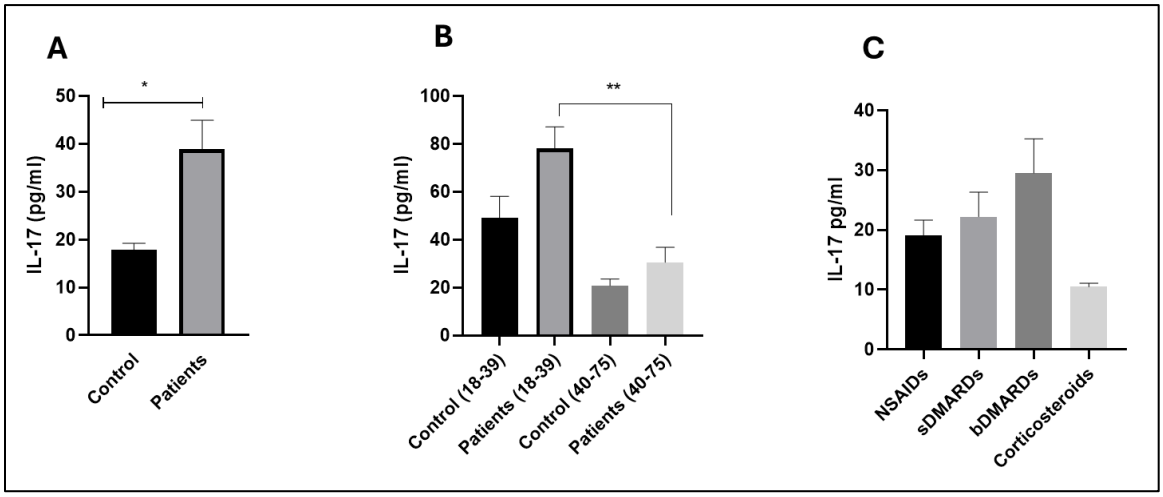

Figure 4. Mean ± S.E. serum IL-10 concentrations in rheumatoid arthritis patients compared with the control group

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Macrophage polarization to pro-inflammatory property

Result showed that there was a significantly higher interleukin 17 (IL-17) level in rheumatoid arthritis patients compared to control group, regardless of age (P-value =0.018) (Figure 5 A). Descriptive statistics of serum IL- 17 levels were shown that mean of IL-17 was higher in rheumatoid arthritis (38.87± 6.05) compared to control group (17.87 ± 1.35) (Table 3).

Interestingly, there were a tendency to be significant differences in IL-17 level in rheumatoid arthritis patients who were eighteen to thirty-nine years old compared to patients who were forty to seventy-five years old (P-value = 0.001), but there were no significant differences when compared to control group (P-value = 0.447) (Figure 5, B). Descriptive statistics of serum IL- 17 levels were shown that mean was higher in RA patients aged 18 to 39 (78.02± 9.08) and 40 to 75(30.53 ± 6.303) when compared to control group (49.87 ± 8.9) (20.75 ± 2.746) (Table 2).

To investigate IL-17 level in patients' treatment response for the four medications as follows: NSAIDs, bDMARDs, sDMARDs, and corticosteroids. There was no significant difference in treatment groups but the patients who were treated with corticosteroids were more likely to have lower levels of IL-17 than other treatments (P-value = 0.736) (Figure 5 C). Descriptive statistics of serum IL- 17 levels were shown that mean of patients who received bDMARDs (29.51 ± 5.754) was more compared to other RA treatments (Table 2).

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Table 2. The Mean ± S.E. IL-17 concentration in the sera of participants (patients compared with the control group)

IL-17 (pg/ml)	Mean ± S.E. Mean	P-value
Control	17.87 ± 1.35	0.018
Patients	38.87± 6.05	
Control (18 - 39)	49.87 ± 8.9	0.447
Patients (18 - 39)	78.02± 9.08	
Control (40 - 75)	20.75 ± 2.746	0.731
Patients (40 - 75)	30.53 ± 6.303	
Patients (18 - 39)	78.02± 9.08	0.001
Patients (40 - 75)	30.53 ± 6.303	
NSAIDs	19.07 ± 2.568	0.995
sDMARDs	22.23 ± 4.093	
bDMARDs	29.51 ± 5.754	0.736
Corticosteroids	10.49 ± 0.5848	

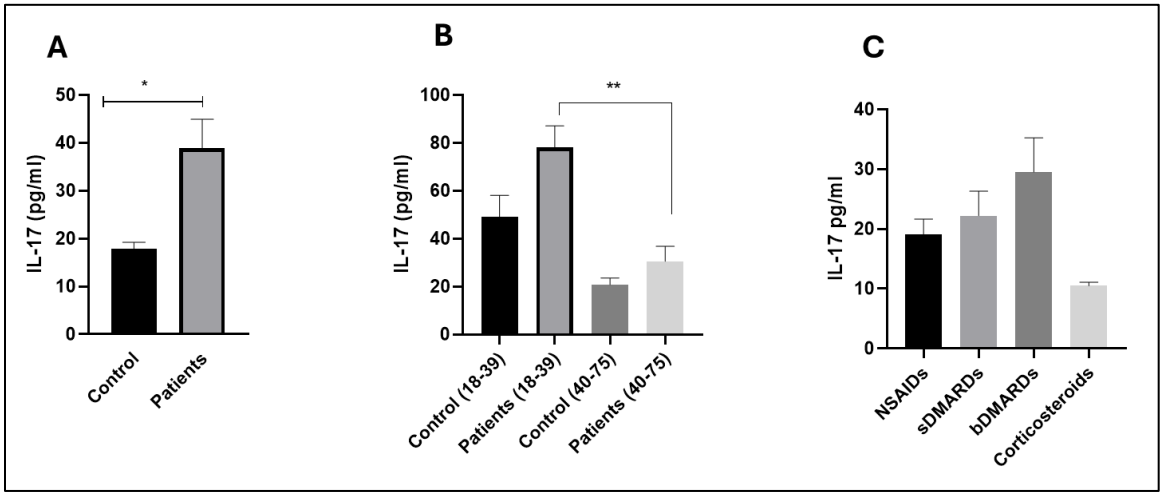

Figure 5. Mean ± S.E. serum IL-17 concentrations in rheumatoid arthritis patients compared with the control group

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Complement response in Rheumatoid arthritis patients

Regardless of age, findings demonstrated that RA patients had a noticeably higher but not significant level of C5a than control group (P-value =0.12) (Figure 6 A). Descriptive statistics of serum C5a levels were shown that mean was higher in rheumatoid arthritis (220.3 ± 19.75) compared to control group (178.0± 17.14) (Table 3).

It is interesting to note that RA patients aged 18 to 39 tended to have higher but not significant C5a levels than control samples of the same age group (P-value = 0.959), and no significant differences were also observed between RA patients aged 18–39 years and those aged 40–75 years (P-value = 0.8939). Furthermore, among individuals aged 40 to 75, the tendency for C5a levels were higher but not significantly different in RA patients than in control subjects (P-value =0.769) (Figure 6 B). Noteworthy, Descriptive statistics of serum C5a levels were shown that mean was higher in rheumatoid arthritis aged 18 to 39 (241.3 ± 53.14) and 40 to 75(212.3 ± 18.71) compared to control (205.6± 37.51) (162.7± 16.43)(Table 3). To investigate patients' treatment effect on C5a level, the corticosteroid treatment had a tendency to have a lower but not significant C5a level compared with other treatments (P-value =0.264) (Figure 6 C). Descriptive statistics of serum C5a level was shown that mean (104.0 ± 8.991) of patients who received corticosteroids was less compared to other RA treatments (Table 3).

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Table 3. The Mean ± S.E. of C5a concentration in the sera of participants (patients compared with the control group). *P <0.05 (adjusted P-values).

C5a ((pg/ml)	Mean ± S.E. Mean	P-value
Control	178.0 ± 17.14	0.12
Patients	220.3 ± 19.75	
Control (18 - 39)	205.6 ± 37.51	0.959
Patients (18 - 39)	241.3 ± 53.14	
Control (40 - 75)	162.7 ± 16.43	0.769
Patients (40 - 75)	212.3 ± 18.71	
Patients (18 - 39)	241.3 ± 53.14	0.8939
Patients (40 - 75)	212.3 ± 18.71	
NSAIDs	272.8 ± 47.91	0.978
sDMARDs	245.2 ±39.75	
bDMARDs	230.1 ± 25.14	0.264
Corticosteroids	104.0 ± 8.991	

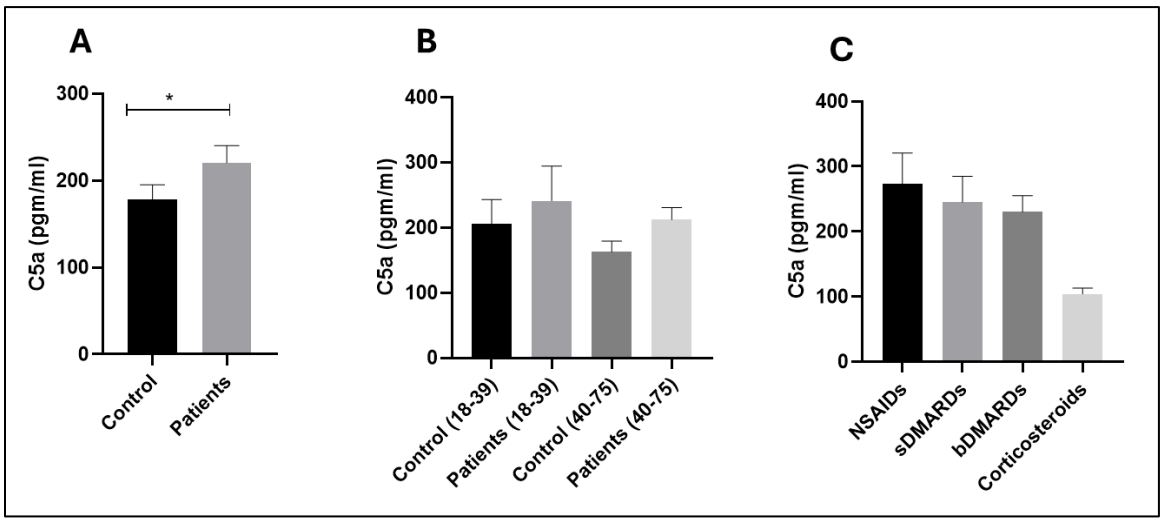

Figure 6. Mean ± S.E. serum C5a concentrations in rheumatoid arthritis patients compared with the control group.*P <0.05 (adjusted P-values)

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Serum Anti-CCP Levels in Rheumatoid Arthritis and Controls

Rheumatoid arthritis patients (age regardless) had significantly higher anti-CCP levels compared to control people (P-value = 0.01)(Figure 7 A). Descriptive statistics of serum Anti--CCP levels were shown that mean (1327 ±201.1) was higher in rheumatoid arthritis compared to control group (4.754 ± 0.4832) (Table 4).

Interestingly, there were no significant differences in anti-CCP levels among RA patients who were eighteen to thirty-nine years old compared to patients who were forty to seventy-five years old (P-value = 0.998), but significant differences were found when compared to the control group (P-value = 0.006). (Figure 7 B).

It was noted that that RA patients aged 18 to 39 had higher Anti-CCP levels than control samples of the same age group. Furthermore, among individuals aged 40 to 75, Anti-CCP levels were significantly higher in RA patients compared to control subjects (P-value = 0.0001) (Table 5).

To investigate patients' treatment for RA patients as follows: NSAIDs (non-steroidal anti-inflammatory drugs) biologic disease-modifying antirheumatic drugs (bDMARDs) Synthetic disease-modifying anti-rheumatic drugs (sDMARDs) and corticosteroids. Corticosteroids, as sometimes mixed with other treatments, had a tendency to have a significantly lower anti-CCP level compared with other treatments (P-value =0.0001) (Figure 7 C). Descriptive statistics of serum C5a level was shown that mean (1943 ± 43.00) of patients who received NSAIDs was less compared to other RA treatments (Table 4).

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Table 4. Descriptive statistics of serum levels anti-CCP antibodies

anti-CCP (U/ml)	Mean ± S.E. Mean	P-value
Control	4.754 ± 0.4832	0.01
Patients	1327 ±201.1	
Control (18 - 39)	3.500 ± 0.000	0.006
Patients (18 - 39)	1189 ±242.8	
Control (40 - 75)	3.865 ± 0.3650	0.0001
Patients (40 - 75)	1238 ± 221.0	
Patients (18 - 39)	1189 ±242.8	0.998
Patients (40 - 75)	1238 ± 221.0	
NSAIDs	1943 ± 43.00	0.0001
sDMARDs	1173 ± 188.2	
bDMARDs	1216 ± 259.5	
Corticosteroids	563.0 ± 202.1	

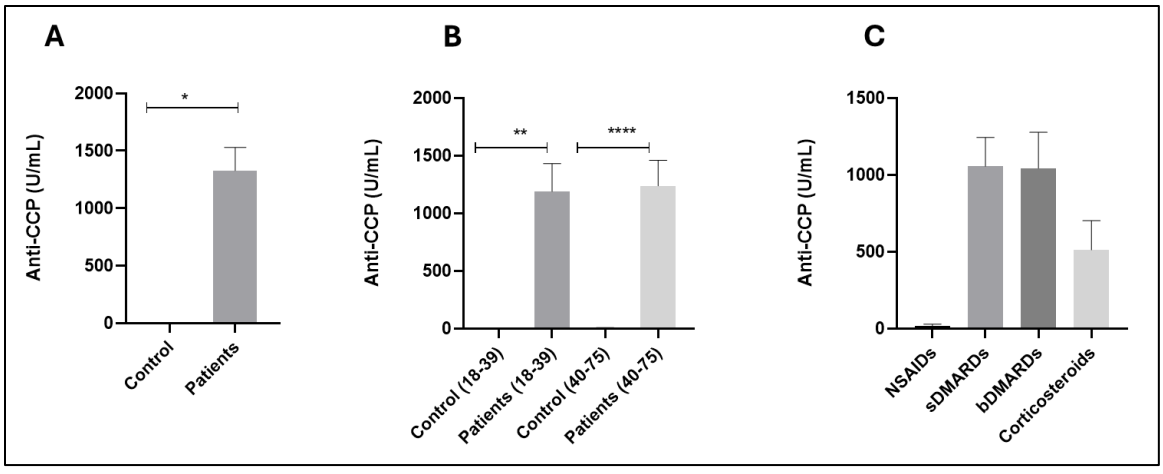

Figure 7. Anti-CCP investigation as a marker for Rheumatoid arthritis disease diagnoses

Mean ±SEM from duplicate determinations is used to present the results. *P <0.05, **P <0.01, ***P <0.005, ****P <0.0001 (adjusted P values).

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Discussion

In the current study, the anti-inflammatory cytokine IL-10 was found in lower concentration in RA patients than in the control population. Conversely, the pro-inflammatory cytokine IL-17 and the complement component C5a were substantially higher in RA patients than in healthy patients. Additionally, among our RA patients, 71.67% were seropositive, while 28.33% were seronegative for the anti-CCP test. All 40 healthy controls tested negative. These results, in turn, support the fact that anti-CCP antibodies can serve as a highly specific biomarker for the diagnosis and prognosis of RA.

In the present study, IL-10 levels of the serum demonstrated a downward movement in the patients of rheumatoid arthritis in contrast to control individuals regardless of age. Previous studies show mixed results on the concentrations of IL-10 in RA patients. Certain studies have indicated high serum IL-10 and that there was an increase in IL-10-producing T cells (CD4+) in active disease indicating a compensatory anti-inflammatory mechanism (16). Conversely, other studies reported significantly lower levels of IL-10 implying that its production was inhibited in RA. The decreased IL-10 levels in the RA patients especially the subgroups of 18-39 and 40-75 years are consistent with the study conducted by (Qu CH, et al) (17). Despite the presence of IL-10 in the RA synovial fluid, it also had inadequate levels to counter the pro-inflammatory cytokines. IL-10 is primarily expressed in higher distribution of T cells and macrophages in synovial tissue, yet expression of the IL-10 receptor on dendritic cells was suppressed by pro-inflammatory cytokines such as TNF-alpha and IL-1. The bioactivity and IL-10 signaling in macrophages were inhibited by chronic exposure to these cytokines, which maintained inflammation in RA (18).

The findings demonstrated that the serum levels of IL-10 were increased in RA patients under bDMARDs treatment, whereas RA patients under corticosteroid treatment had lower IL-10 levels. Although these findings were not consistent with previous studies, they were logical and scientifically valid in view of the heterogeneity and complexity of RA (19).

IL-17 is also a major player in the pathogenesis of RA by connecting longstanding inflammatory processes and degradation of joints. IL-17, mainly secreted by Th17 cells, initiates the infiltration and activation of osteoblasts, macrophages, and fibroblast-like synoviocytes (FLS) (20).

Throughout RA, IL-17 levels are commonly elevated at an early stage prior to clinical manifestation and then sustained by feedback mechanisms with

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IL-6 and IL-22. The mechanisms outline the dual nature of IL-17 in the initiation and maintenance of synovial inflammation and bone destruction in RA (4).

Elevated IL-17 levels were observed in the RA patients in general. Patients 18-39 years of age showed a lower level of IL-17 compared to controls, and older patients 40-75 years of age showed an increased level, perhaps as a result of immune mechanisms and inflammation differentials. In RA, levels of IL-17 were strongly linked to age. These findings indicated that corticosteroids that are commonly used in combination with other medications were likely to reduce IL-17 levels as opposed to NSAIDs, bDMARDs, or sDMARDs, probably because they have an anti-inflammatory effect in suppressing T cells, like the Th1 and Th17 cells (21).

However, the data indicated that the concentration of IL-17 varied among RA patients. Serum IL-17 levels in certain RA patients were noticeably higher (214.60 pg/ml), whereas those in other RA participants were noticeably lower (3.3 pg/ml). This variation in IL-17 among RA participants may be explained by the heterogeneous nature of RA, and the amount of IL-17 can be impacted by a number of variables, such as age, IL-17 gene polymorphisms (22). Immune cell profile, stage and activity of the disease, and concurrent disorders like ischemic cardiovascular disease that canraise IL-17 levels (23). Body composition may also play a role, as a study of rheumatoid arthritis patients attending the same Rizgary Teaching Hospital in Erbil found that higher body mass index was significantly associated with greater disease activity, measured by DAS28 and CDAI (36). Increased IL-17 levels were also documented among RA patients who were smokers or those with vitamin D deficiency (24). Numerousprior researches have demonstrated that RA patients exhibited a higher level of IL-17 than the control groups, which aligned with the results of our present research (25). The steady rise in serum IL-17 levels during RA patient investigations was demonstrated to be closely associated with joint degradation and disease activity (26).

C5a is a powerful anaphylatoxin produced by stimulation of the complement system and has been shown to represent the key component in the immunopathology of rheumatoid arthritis (RA) (27). Its activity may be affected by genetic variants and environmental exposure, such as infection (28). Numerous earlier studies have shown that RA patients exhibited a high level of C5a in serum, plasma, and synovial fluid, where its level correlated with DAS 28 and the presence of P. gingivalis antibodies (28). Our discoveries indicated that C5a levels were significantly raised in all age groups of RA patients when compared

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to controls. In RA, the level of C5a goes up due to the fact that RA is a chronic autoimmune inflammatory disease whereby immune complexes and inflammation engage the complement system, leading to higher production of cleavage products such as C5a (11).

Additionally, corticosteroid-treated RA patients were measured lower concentrations of C5a when compared with other medications, which mean that the type of treatment affected the level of C5a in serum. Corticosteroids in RA can reduce C5a through inhibitions of complement activity, such as CRP suppression and the influences related to lipopolysaccharides (29). These outlined roles were consistent with our study findings.

One of the most specific indicators of rheumatoid arthritis (RA) is anti-CCP antibodies. As a predictive tool, anti-CCP antibodies are also helpful in the early diagnosis of the disease, which occurs many years before the disease's actual clinical manifestations (30).

These results suggested that anti-CCP antibodies were much more abundant among RA patients, irrespective of their age, and that all healthy individuals were negative to this autoantibody. Moreover, age did not affect the elevation of anti-CCP antibodies among RA patients. These findings highlighted the important role of anti-CCP antibodies as a highly specific prognostic and diagnostic biomarker in RA. During immune dysregulation or inflammatory conditions such as those found in RA patients, neutrophil extracellular traps (NETs) which are the main source of citrullinated protein were elevated in the circulation and synovial fluid compared to healthy individuals. Citrullinated proteins are more immunogenic and have a higher affinity for the HLA-DR antigen-binding groove when compared to native proteins thereby stimulating the immune system to produce high amount of anti-CCP antibodies (31). This strategy helped to clarify why control groups were negative to anti-CCP test.

Numerous earlier studies have shown that the serum of RA patients had higher concentrations of anti-CCP antibodies. Anti-CCP antibodies are a highly specific biomarker for the diagnosis of RA as well as most RA patients are seropositive for this marker (32). Those results were consistent with our current study's findings.

Earlier studies with regard to the effect of therapeutic interventions on anti-CCP antibody levels in RA patients have had mixed outcomes. This discrepancy could be connected with the variance in follow-up period, dose, disease activity, and technique of anti-CCP analysis. Nonetheless, the majority of earlier research suggested that bDMARDs were associated with a decrease in serum

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anti-CCP antibodies (33). Drugs such as tocilizumab, adalimumab, infliximab, etanercept, and other bDMARDs may decrease anti-CCP levels by regulating the activity of B cells and restraining inflammation, which promotes autoantibody synthesis (34). Whereas, as opposed to several previous studies, some other studies showed that corticosteroids have been demonstrated to decrease anti-CCP in RA since they decrease citrullination and the production of PAD4 enzymes in synovial tissue in some studies. Corticosteroids are faster-acting than methotrexate, perhaps through blocking the NF-kB pathway that controls expression of PAD4, which could reduce anti-CCP levels in treated patients (10).

According to the finding of this study, corticosteroids, which are frequently used as combination therapy with other medications, were associated with reduced anti-CCP in RA patients. Nonetheless, it cannot be concluded that corticosteroids are more effective than bDMARDs, as co administration with NSAIDs, bDMARDs, or sDMARDs may produce a synergistic effect in reducing anti-CCP levels.

Conclusion

Our study demonstrated that macrophages from rheumatoid arthritis patients transitioned from an anti-inflammatory phenotype, characterized by IL-10, to a pro-inflammatory phenotype, marked by IL-17. Patients with rheumatoid arthritis had lower levels of anti-inflammatory cytokine markers like IL-10 and greater levels of pro-inflammatory cytokines like IL-17, inflammatory markers like C5a, and rheumatoid arthritis markers like anti-CCP, all of which are signs of immune regulatory problems. The incorporation of these indicators reveals a novel comprehension of macrophage polarization, complement activation, and therapy responses in the etiology of rheumatoid arthritis, facilitating disease monitoring.

Relevance to the Sustainable Development Goals (SDGs)

This study aligns with the National Sustainable Development Goals (SDG) by addressing key scientific challenges. Specifically, the research contributes to SDG 3 (Good Health and Well-Being).

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interest

The authors declare that they have no competing interests.

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