Association between the CCR6/CCL20 axis and IL-17A level in patients with rheumatoid arthritis

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Abstract. Rheumatoid arthritis (RA) is a common systemic autoimmune disease characterized by chronic inflammation. Accumulating evidence has established the pivotal role of the C-C chemokine receptor type (CCR)6-chemokine ligand 20 (CCL20) axis in the recruitment of IL-17A to the arthritic joint. The aim of the present study was to investigate the association between the CCR6/CCL20 axis and IL-17A production in patients with RA. The present study included 120 subjects aged 30-65 years, 20 patients diagnosed as patients with RA who formerly did not receive steroids or any immune-modulatory therapy, 30 patients on methotrexate (MTX) treatment, 30 patients receiving MTX + biological therapy and 40 healthy controls. ELISA was used to quantify the expression levels of CCR6, CCL20 and IL-17A. The salivary concentration levels of IL-17A, CCR6 and CCL20 were increased in patients with RA compared with those of the control subjects. Furthermore, the average salivary concentration levels of the biomarkers were markedly elevated in the newly diagnosed RA and in the MTX groups compared with those noted in the MTX + biological therapy group. By contrast, a significant decrease was noted in the mean ratio of CCR6/CCL20 among the patient groups in comparison to the control group. Furthermore, a substantial positive association was noted between the expression levels of IL-17A and CCR6 in the newly diagnosed RA group, as well as between CCR6 and CCL20 in the MTX and newly RA groups, while a positive significant correlation ($P<0.05$) was noted between the expression levels of IL-17A and CCL20 in each of the control groups, the MTX and the MTX + biological groups. On the whole, newly diagnosed RA indicated high levels of salivary biomarkers and a strong positive association was noted between the high expression levels of the chemokines CCR6 and CCL20 and the increased IL-17A levels noted in the patients.

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Introduction

Rheumatoid arthritis (RA) is characterized as a systemic autoimmune disorder linked to a persistent inflammatory process that can harm both joints and extra-articular organs (1). Initially, only a limited number of joints are involved; however, in the advanced stages, multiple joints are affected and extra-articular symptoms frequently occur, with a prevalence of 0.4-1.3% in the population; this percentage is influenced by sex (women are affected two to three times more often than men) and age (the incidence of new RA diagnoses peaks in the sixth decade of life). Affected joints exhibit erythema, edema and an increased temperature, ultimately leading to muscular atrophy in the surrounding region. The clinical progression and severity of RA significantly differ among patients. Individuals with progressive active illness experience joint degeneration and deformity over time, which may include subcutaneous nodules and swan-neck deformities. RA can reduce life expectancy by ∑ 5 to 10 years (2). A significant long-term consequence of RA is a pronounced elevation in the risk of cardiovascular disease (3). Although the etiology and progression of RA remain incompletely elucidated, various therapeutic modalities are accessible, significantly altering the prognosis of patients with the disease (4). Various cell types are implicated in the pathophysiology of RA, including synovial fibroblasts, osteoclasts, immune-associated T- and B-lymphocytes, and macrophages. The orchestration of these cells induces the release of diverse inflammatory mediators (cytokines and chemokines) that perpetuate the chronic inflammatory response of the disease. Chemokines and their receptors regulate lymphocyte recruitment to inflamed joints in RA. Cytokines, encompassing both pro-inflammatory and anti-inflammatory types, are recognized for their essential involvement in the evolution of RA via inflammation and the degradation of articular cartilage (5-8). Chemokine receptors constitute a family of G protein-coupled receptors (GPCRs) modulated by low molecular weight protein-ligands termed chemokines. These molecules feature a globular core structure maintained by 1-2 conserved disulfide bridges, which are crucial for leukocyte trafficking via the establishment of chemotactic gradients. Chemokines and their receptors are crucial in various physiological and pathological processes that regulate the activation, migration, differentiation and survival of leukocytes and other hematopoietic cells (9-11). The chemokine receptor (CCR)6 is a class A GPCR within

the chemokine family, noted for its notable therapeutic promise in immunological research (12). CCR6 is expressed in various cell types, including B cells, immature dendritic cells, innate lymphoid cells, Langerhans cells, neutrophils, regulatory T cells and T helper (Th) 17 cells (13). The sole chemokine ligand for CCR6 is chemokine ligand 20 (CCL20), which is also referred to as macrophage inflammatory protein (MIP)-3α, Exodus-1 and liver and activation regulated chemokine. In humans, it is expressed by neutrophils, Th17 cells and peripheral blood mononuclear cells. This axis has distinct functions in immunological homeostasis and activation (13-15). In patients with RA, $CCR6^+$ Th cells are present in inflamed synovium and elevated levels of peripheral blood $CCR6^+$ Th cells are observed in individuals with early-stage RA. The characterization of $CCR6^+$ Th cells demonstrates a pathogenic profile, characterized by pro-inflammatory cytokine production, and *in vitro* investigations have indicated the robust pathogenic activity of $CCR6^+$ Th cells derived from individuals with RA (16,17). Synovial T-lymphocytes generate cytokines, such as TNFα, IFNγ and IL-17A (18). The production of pro-inflammatory cytokines was originally ascribed to Th1 cells (18). Subsequently, it was elucidated that IL-17A production among Th cells was confined to a distinct Th cell subpopulation, subsequently designated as Th17 (19). Significant advancements have been achieved in comprehending the intricacies of Th17 biology throughout the past decade of research in RA. The upregulation of the CCR6/CCL20 axis in the synovial tissues and salivary glands (in cases of secondary Sjögren's syndrome) is considered to contribute to the recruitment of Th17 cells, which in turn enhances IL-17A production and promotes the inflammatory cycle (20). The interaction between CCR6 and CCL20 is critical, not only for the migration of Th17 cells, but also for their activation and differentiation. CCL20 has been shown to be involved in the differentiation of naive T-cells into Th17 cells, thereby directly influencing IL-17A production in patients with RA (21). Numerous mediators of inflammation, collagen degradation and/or bone remodeling exhibit significant alterations in the blood of individuals with rheumatic illnesses. Given that saliva comprises numerous serum-derived mediators, it may serve as a valuable tool for monitoring rheumatic diseases (22). The present study notably examined the regulatory effects of the salivary CCR6/CCL20 axis on IL-17A production in RA.

Patients and methods

Study participants. The present study was designed as a case-control study including 120 subjects divided into 40 healthy subjects and 20 patients diagnosed as patients with RA who formerly did not receive steroids or any immune-modulatory therapy and 60 patients diagnosed as patients with RA who were receiving therapeutic agents for RA [30 patients receiving methotrexate (MTX), and 30 patients receiving MTX and biological therapy (etanercept)]. These patients were recruited from attendees seeking treatment at the Rheumatology Patient Clinic at Baghdad Teaching Hospital (Baghdad, Iraq) for a period from November, 2023 to Abril, 2024.

A comprehensive case sheet gathered data on the name, age, sex and overall health status of each patient. The exclusion criteria included patients with RA with other autoimmune or systemic inflammatory diseases, smokers or alcoholic patients and pregnant women. Ethical considerations were obtained from a scientific committee affiliated with the College of Dentistry at Baghdad University (reference no. 863 in November, 2023). In order to acquire an informed consent, all participants were requested to provide their approval for the collection of saliva samples.

The sample size was calculated utilizing G Power 3.1.9.7 (developed by Franz-Faul, University of Kiel, Germany), with a study power of 95%, an alpha error probability of 0.05, employing a two-sided statistical test, specifically a two independent samples t-test and presuming an effect size of 0.8 (large) between the two groups under these parameters. The sample size comprised 120 individuals.

Collection of saliva samples. Unstimulated saliva was collected from participants by passive drooling into a plastic cup, without any stimulation or spitting, yielding ∑ 3 ml saliva from each participant. Saliva was extracted from a disposable cup using a micropipette, transferred to a sterile plain tube and centrifuged at 2,000 x g for 3 min at 4˚C to isolate the clear supernatant, which was subsequently aspirated into Eppendorf tubes and stored in a freezer at -20˚C until the day of analysis.

Assessment of the levels of IL-17A, CCR6 and CCL20. Saliva samples were examined for salivary biomarkers with commercially available ELISA kits for humans, adhering to the manufacturers' protocols for IL-17A (cat. no. SEA063Hu from Cloud-Clone Corp.) and CCL20 (cat. no. SEA095Hu from Cloud-Clone Corp.) and CCR6 (cat. no. LS-F19045 from LS Bio).

Statistical analysis. Data description, analysis and presentation were conducted utilizing SPSS (IBM Corp.; version 22). The descriptive analysis included the mean and standard deviation, whereas percentage and frequency pertained to qualitative variables. The Pearson's correlation parametric test was employed to evaluate the correlation between two quantitative variables. The Shapiro-Wilk test was employed to verify the normality of the distribution. Homogeneity was confirmed by one-way analysis of variance, employing the Tukey's test to assess differences among multiple groups. Multiple pairwise comparisons of CCL20/CCR6 were conducted using the Games-Howell post hoc test. Two qualitative variables and their distributional associations were analyzed using the Chi-squared test.

Results

The mean age of the patients newly diagnosed with RA was 46.73±13.36 years and that of patients on MTX treatment was 47.43±11.69 years; in the MTX + biological therapy group, the mean age of the patients was 43.86±11.20 years, as compared to control group which was 41.05±6.27 years. Furthermore, there was a female predominance among patients; the ratio was 2:1. In addition, no significant differences ($P>0.05$) were noted in the mean levels of age, disease duration and the onset of treatment and the number of participants among the study groups, as shown in Table I and Fig. 1.

Table I. Demographic features of the patients and control groups involved in the present study.

Demographic characteristics	Study groups				P-value
	Control group, n=40	Newly diagnosed RA group, n=20	MTX RA group, n=30	MTX + biological therapy RA group, n=30	
Age (years), mean ± SD	41.05±6.27	46.73±13.36	47.43±11.69	43.86±11.20	0.060 (NS)[a]
Disease duration, mean ± SD			2.707±1.341	3.183±1.021	0.127 (NS)[b]
Onset of treatment, mean ± SD			1.933±1.150	2.433±0.926	0.067 (NS)[b]
Sex, n (%)					
Female	19 (48.72)	13 (68.42)	21 (70)	20 (66.67)	0.230 (NS)[c]
Male	21(51.28)	7 (31.58)	9 (30)	10 (33.33)	

The female/male ratio was 2:1. Data were analyzed using [a]ANOVA, [b]an unpaired t-test, or [c]Chi-squared test. There were no statistically significant differences (P>0.05) in age, disease duration, onset of treatment and number of participants among the study groups. RA, rheumatoid arthritis; MTX, methotrexate; NS, not significant; SD, standard deviation.

The present study demonstrated a substantial elevation (P<0.05) in the mean salivary levels of IL-17A, CCR6 and CCL20 across all patient groups relative to the control group, as illustrated in Table II and Fig. 2.

Moreover, the results revealed a significant increase in the mean levels of IL-17A in the newly diagnosed patients and those on MTX therapy, as compared with those of the control group (P<0.05). Conversely, a significant decrease (P<0.05) was noted in the mean levels of IL-17A between individuals undergoing MTX treatment and those receiving MTX + biological therapy in comparison to the newly diagnosed RA patient groups, as shown in Tables II and III.

The present study also demonstrated a significant increase in the mean CCR6 levels in the patient groups (newly diagnosed RA patient group and MTX treatment groups), as compared with the control group. However, there was a significant decrease in the mean CCR6 levels (P<0.05) in the MTX and MTX + biological groups as compared to the newly diagnosed group and a significant decrease (P<0.05) in MTX + biological group as compared to MTX group, as shown in Tables II and IV.

The levels of CCL20 exhibited a significant increase (P<0.05) in the newly diagnosed and MTX groups, as compared to control group. However, there was a significant decrease in the levels of CCL20 (P<0.05) in the MTX and MTX + biological groups, as compared to the newly diagnosed group, as shown in Tables II and V.

In addition, the present study indicated a significant decrease (P<0.05) in the mean ratio of CCL20/CCR6 among the patient groups (newly diagnosed RA, MTX treatment group and MTX + biological therapy) compared with that of the control group, as shown in Table VI.

There was a significant increase (P<0.05) in the CCL20/CCR6 ratio in patients in the MTX and MTX + biological groups compared with newly diagnosed groups, and the MTX group compared to the MTX+ biological group. In addition, the present study revealed a significant decrease (P<0.05) in the mean ratio of CCL20/CCR6 among the patient groups (newly diagnosed RA and MTX) compared with that of the control group. There was no significant difference in the mean ratio of CCL20/CCR6 between the MTX + biological group and the control group (Tables VI and VII).

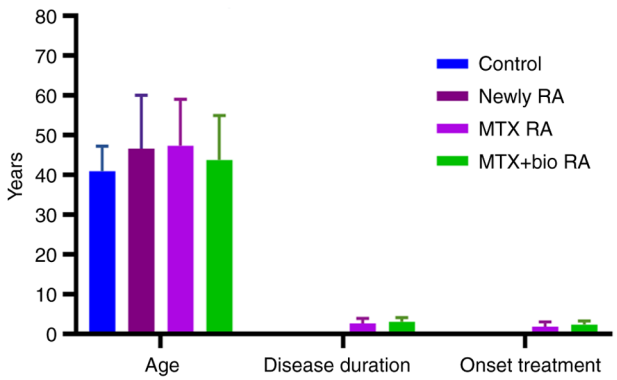

Figure 1. Mean levels of disease duration and onset of treatment in the MTX patient group and in the MTX + biological therapy group. MTX, methotrexate.

In addition, the present study revealed a significant positive correlation (P<0.05) between IL-17A and CCL20 in the control, MTX and MTX + biotherapy groups. By contrast, a significant positive correlation (P<0.05) was noted between the IL17A and CCR6 levels in the newly diagnosed group. A significant positive correlation (P<0.05) was also noted between the CCR6 and CCL20 levels in the newly diagnosed RA groups and MTX group (Table VIII). The aforementioned correlations are illustrated as scatter plots in Figs. 3-6.

Discussion

Previous studies have indicated that the typical patient age is between the fourth and fifth decade of life (23,24). In addition, the present study indicated that the prevalence of RA was higher in females than in males; these findings are consistent with the findings reported in the study by Bukhari *et al* (25). According to multiple research studies conducted in Iraq (26-28), females were more likely than males to suffer from RA. According

Table II. Salivary mean level of IL-17A, CCR6 and CCL20 in the study groups.

Salivary biomarker	Study groups				P-value
	Control group, n=40	Newly diagnosed RA group, n=20	MTX group, n=30	MTX + biological therapy group, n=30	
IL-17A (pg/ml)	145.04±23.45	223.85±38.77	174.14±46.74	145.04±38.24	0.001[a]
CCR6 (pg/ml	670±200	1960±370	1400±350	860±310	0.001[a]
CCL20 (pg/ml)	98.05±23.37	141.68±24.78	118.29±38.87	111.13±22.43	0.001[a]

The present study demonstrated a substantial elevation (P<0.05) in the mean salivary levels of IL-17A, CCR6 and CCL20 across all patient groups relative to the control group. Data were analyzed using ANOVA. [a]Indicates a statistically significant difference (P<0.05). CCR6, C-C chemokine receptor type 6; CCL20, chemokine ligand 20; MTX, methotrexate.

to the findings of the present study, the newly diagnosed RA group exhibited higher mean levels of IL-17A than those of the control, MTX and MTX + biological therapy groups. This was in line with a previous study reported by Hemdan *et al* (29), which indicated an imbalance in the cytokine expression levels, with IL-17A levels being higher in RA than in control subjects. Atwa *et al* (30) indicated that the levels of IL-17 were significantly increased in cases with RA compared with those of healthy controls. By contrast, Moran *et al* (31) discovered that IL-17A was strongly expressed in the inflammatory joint, driving disease activity in RA. Therefore, based on these findings, the data indicate the importance of IL-17A in the course of RA, highlighting their potential for prognosis and disease monitoring.

Furthermore, in the present study, an increase in the IL-17A mean value was noted in patients newly diagnosed with RA and in patients in the MTX treatment group compared with the control group, with highly significant differences. This is consistent with the results reported in the study by Roşu *et al* (32), indicating an increase in the average IL-17A value in patients newly diagnosed with RA and in patients with MTX treatment groups compared with the control group, which was highly significant.

By contrast, in the present study, a decrease was noted in the mean levels of IL-17A in the MTX + biological therapy group compared with those of the MTX treatment groups. This is consistent with the findings of the study by Lina *et al* (33), indicating a significant decrease in the levels of IL-17 among the combined therapy group.

As regards the mean levels of salivary CCR6, the results presented herein indicated a significant increase in the mean value of CCR6 among the study groups (newly diagnosed RA and MTX treatment group) compared with those of the control group. This is consistent with the study conducted by Cheng *et al* (34), which demonstrated a significant increase in the levels of CCR6 in patients with RA compared with those of the control group. As previously reported by Komatsu and Takayanagi (35), in patients with early-stage RA, increased levels of CCR6 were noted in the synovium. By contrast, a reduction in the average CCR6 levels was demonstrated in patients with MTX treatment and with MTX + biological therapy compared with those of the newly RA groups. The reason is due to the effects of the drugs on the

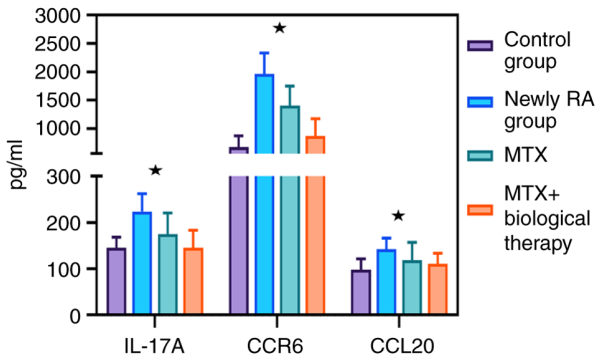

Figure 2. Salivary mean levels of IL-17A, CCR6 and CCL20 in the study groups. There was an increase in the IL-17A mean value in the newly diagnosed patients and patients on MTX treatment group when compared to the control group, with highly significant differences (*P<0.05), whereas a decrease in the IL-17A mean level was detected in patients in the MTX + biological therapy groups when compared to the control groups with no significant difference (P>0.05). There was an increase in the mean CCR6 and CCL20 levels in the group of patients with RA (newly diagnosed patients with RA, MTX treatment and MTX + biological therapy) compared to the control group, with a significant difference (*P<0.05). CCR6, C-C chemokine receptor type 6; CCL20, chemokine ligand 20; MTX, methotrexate.

CCR6 levels in patients with RA. This is consistent with the study by Adams *et al* (36), indicating that MTX treatment resulted in a significant reduction in CCR6 levels in patients with RA, suggesting its ability to modulate the immune response associated with RA.

However, the present study indicated that biological therapy alongside MTX exerted a synergistic effect, resulting in an even greater reduction in CCR6 levels compared with those noted in MTX alone. These results suggest that the combination therapy of MTX and biological agents may provide enhanced therapeutic benefits for patients newly diagnosed with RA, potentially influencing treatment decisions and long-term prognosis. This aligns with the findings of the previous study by Schmalzing *et al* (37), confirming that this combination therapy, specifically MTX + biologic therapy, reduced CCR6 levels, representing an important area of treatment.

In the present study, the CCL20 levels in groups of patients with RA were analyzed and compared with those of the healthy control group; the results indicated that the mean CCL20 levels in the patient groups were significantly elevated

Table III. Intergroup comparisons of the IL-17A (pg/ml) mean values using Tukey's test.

Study groups	Mean difference	P-value
Newly diagnosed vs. control	78.82	0.001[a]
MTX vs. control	29.1	0.0078[a]
MTX + biological therapy vs. control	-0.003	(NS)
Newly diagnosed vs. MTX	-49.71	0.001[a]
Newly diagnosed vs. MTX + biological therapy	-78.82	0.001[a]
MTX vs. MTX + biological therapy	-29.10	0.0141[a]

Please refer to Table II for the mean values of IL-17A. A significant increase in the mean levels of IL-17A was observed in the newly diagnosed patients and those on MTX therapy, as compared with those in the control group (P<0.05). Conversely, a significant decrease (P<0.05) was noted in the mean levels of IL-17A in those undergoing MTX treatment and MTX + biological in comparison to the newly diagnosed patients with RA. In addition, there was a decrease in the IL-17A level in the MTX + biological therapy group vs. the MTX group. [a]Indicates a statistically significant difference (P<0.05). NS, not significant; MTX, methotrexate.

Table IV. Intergroup comparisons of mean values of CCR6 (ng/ml) between all pairs of groups using Tukey's test.

Study groups	Mean difference	P-value
Newly diagnosed vs. control	1.293	0.001[a]
MTX vs. Control	0.731	0.001[a]
MTX + biological therapy vs. control	0.193	0.0543 (NS)
Newly diagnosed vs. MTX	-0.562	0.001[a]
Newly vs. MTX + biological therapy	-1.100	0.001[a]
MTX vs. MTX + biological therapy	-0.538	0.001[a]

Please refer to Table II for the mean values of CCR6. The present study also demonstrated a significant increase in the mean CCR6 levels in the patient groups (newly diagnosed RA patient group and MTX treatment) as compared with the control group, while there was a significant decrease (P<0.05) in the MTX and MTX + biological therapy groups as compared to the newly diagnosed group. Moreover, there was a significant decrease (P<0.05) in MTX + biological therapy group as compared to the MTX group. [a]Indicates a statistically significant difference (P<0.05). NS, not significant; MTX, methotrexate.

Table V. Intergroup comparisons of mean values of CCL20 (pg/ml) using Tukey's test.

Study groups	Mean difference	P-value
Newly diagnosed vs. control	43.64	0.001[a]
MTX vs. Control	20.25	0.0158[a]
MTX + biological therapy vs. control	13.080	0.2107 (NS)
Newly diagnosed vs. MTX	-23.39	0.023[a]
Newly vs. MTX + biological therapy	-30.56	0.0014[a]
MTX vs. MTX + biological therapy	-7.167	0.7449 (NS)

Please refer to Table II for the mean values of CCL20. The levels of CCL20 exhibited a significant increase (P<0.05) in the newly diagnosed and MTX groups as compared to control group, while there was a significant decrease (P<0.05) in the MTX and MTX + biological therapy groups as compared to the newly diagnosed group. [a]Indicates a statistically significant difference (P<0.05). NS, not significant; MTX, methotrexate.

compared with those of the control group. This aligns with the findings of the study by Bonelli *et al* (38), which indicated that CCL20 levels were elevated in patients with RA and afflicted joints, suggesting the significant role of CCL20 in disease pathophysiology. The present study demonstrated a substantial elevation in CCL20 levels in patients newly diagnosed with RA. This aligns with the study by Crijns *et al* (39). The levels of CCL20 were markedly elevated in patients newly diagnosed with RA compared with those of the control group. CCL20 is pivotal in the immune response of subjects with RA and is therefore regarded as a potential biomarker for disease activity (39).

Table VI. Descriptive and statistical analysis of the CCL20/CCR6 ratio among the groups.

	CCL20/CCR6 ratio			
Groups	Minimum	Maximum	Mean ± SD	P-value
Control	27.02	370.23	163.14±73.25	0.001[a]
Newly diagnosed	51.63	88.33	73.10±10.21	
MTX	52.20	155.82	87.36±25.91	
MTX + biological therapy	53.59	302.13	141.35±49.19	

The present study revealed a significant decrease (P<0.05) in the mean ratio of CCL20/CCR6 among the patient groups (newly diagnosed RA, MTX treatment group and MTX + biological therapy) as compared with that of the control group. [a]Indicates a statistically significant difference (P<0.05). MTX, methotrexate.

Table VII. Multiple pairwise comparisons of CCL20/CCR6 using the Games-Howell post hoc test.

Study groups	Mean difference	P-value
Newly diagnosed vs. control	-90.03	0.001[a]
MTX vs. control	-75.77	0.001[a]
MTX+ biological therapy vs. control	-21.78	0.458 (NS)
Newly diagnosed vs. MTX	14.26	0.047[a]
Newly diagnosed vs. MTX + biological therapy	68.25	0.001[a]
MTX vs. MTX + biological therapy	53.98	0.001[a]

There was a significant increase (P<0.05) in the CCL20/CCR6 ratio of patients in the MTX and MTX + biological groups compared with newly diagnosed groups and MTX group compared to MTX + biological therapy group. In addition, the present study indicated a significant decrease (P<0.05) in the mean ratio of CCL20/CCR6 among the patient groups (newly diagnosed RA and MTX) compared with that of the control group. There was no significant difference in the mean ratio level of CCL20/CCR6 between MTX + biological therapy group and control group. [a]Indicates a statistically significant difference (P<0.05). NS, not significant; MTX, methotrexate.

Table VIII. Correlation between salivary biomarkers.

		CCR6		CCL20	
Group	Correlation	R value	P-value	R value	P-value
Control	IL-17A	-0.184	0.261 (NS)	0.639	0.001[a]
	CCR6			-0.228	0.163 (NS)
Newly diagnosed	IL-17A	0.656	0.002[a]	0.214	0.378 (NS)
	CCR6			0.666	0.002[a]
MTX	IL-17A	0.270	0.148 (NS)	0.750	0.001[a]
	CCR6			0.551	0.002[a]
MTX + biological therapy	IL-17A	0.276	0.140 (NS)	0.470	0.009[a]
	CCR6			0.257	0.171 (NS)

The study indicated a significant positive correlation (P<0.05) between IL-17A and CCL20 in the control, MTX and MTX + biotherapy groups. By contrast, a significant positive correlation (P<0.05) was noted between the IL17A and CCR6 levels in the newly diagnosed group. A significant positive correlation (P<0.05) was also noted between CCR6 and CCL20 levels in newly diagnosed RA group and MTX group. R values indicate Pearson's correlation coefficients. [a]Indicates a statistically significant difference (P<0.05). NS, not significant; MTX, methotrexate.

The present study indicated a significant decrease in the mean CCL20/CCR6 ratio between the patient groups (newly diagnosed RA, patients receiving MTX treatment and MTX + biological therapy groups) compared with that of the control group. In the study by Lee and Körner (40), the CCL20/CCR6 axis played a major role in RA, as the inappropriate activation of

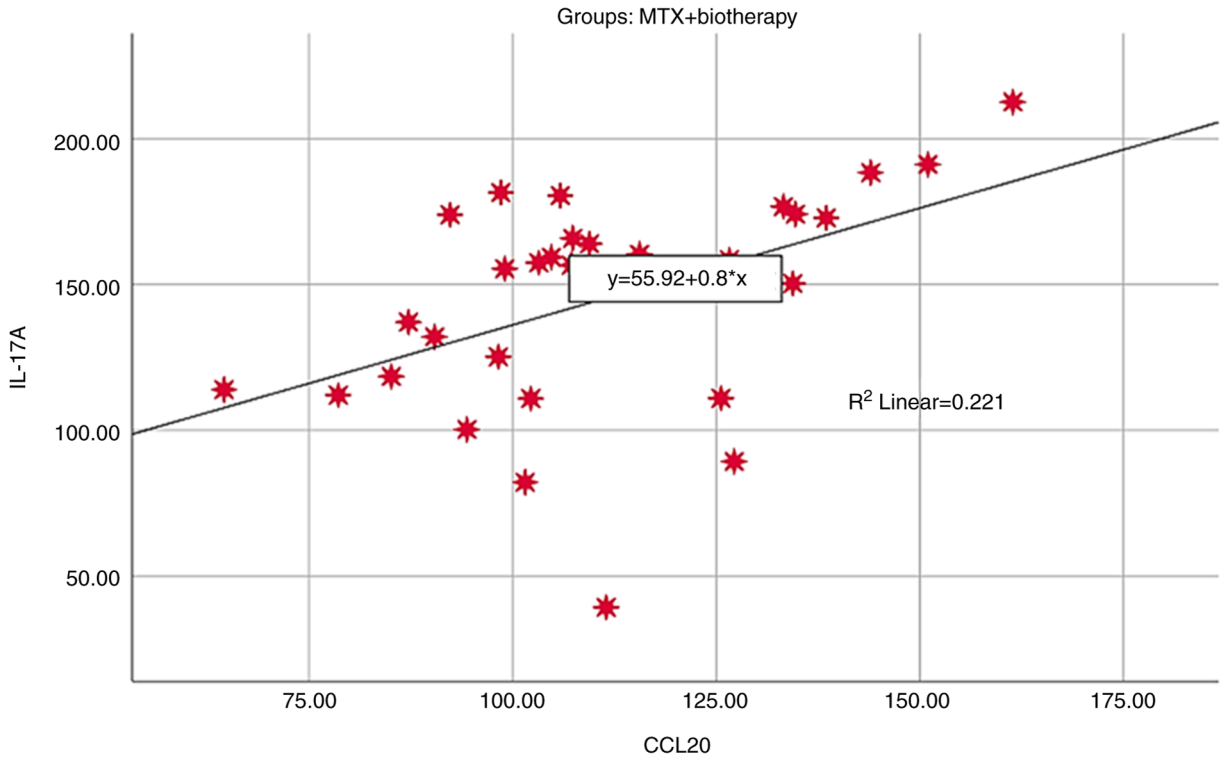

Figure 3. Scatter plot depicting the correlation between the CCL20 and IL-17A levels in the MTX + biological therapy group. Each red asterisk (*) represents a data point for an individual subject in this group. The black line represents the linear regression line fitted to the data. The coefficient of determination (R^2), indicating the proportion of the variance in IL-17A that is predictable from CCL20, is 0.221 for this linear model. CCL20, chemokine ligand 20.

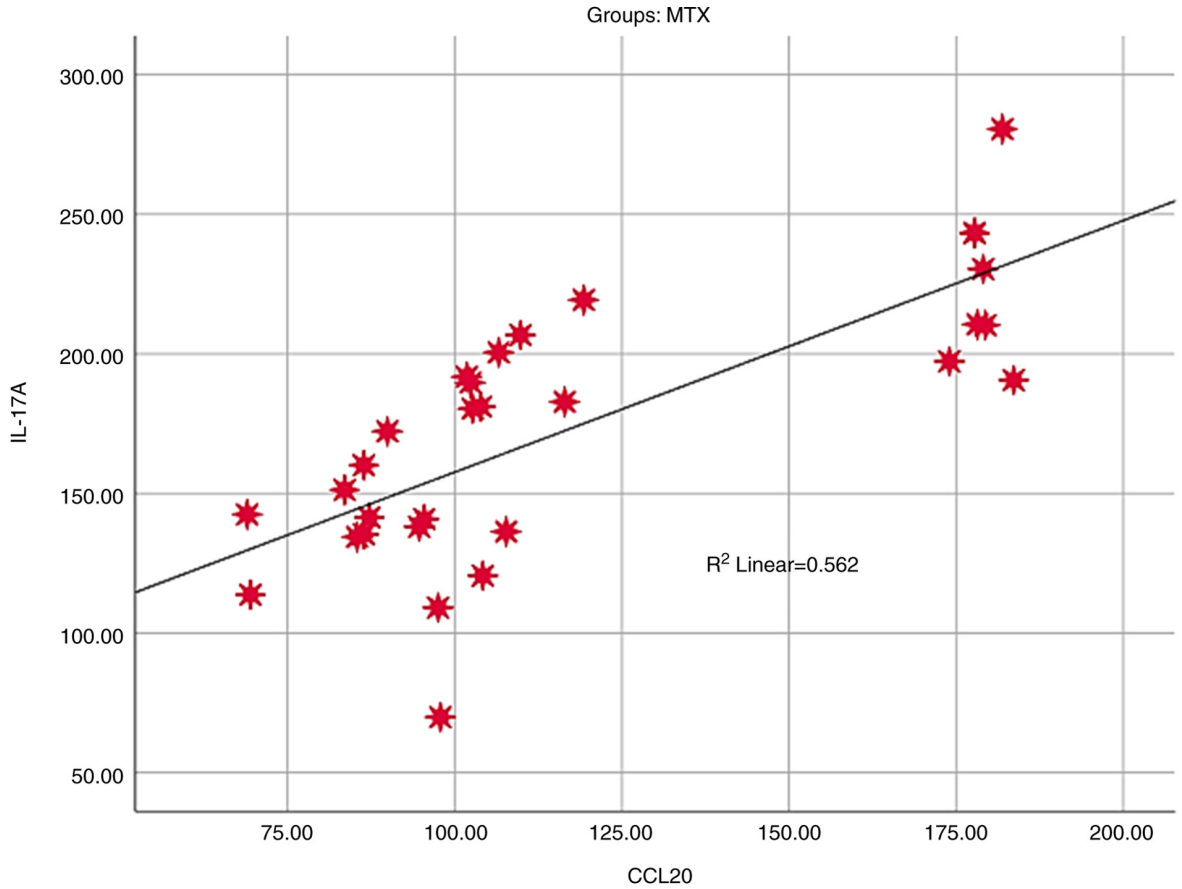

Figure 4. Scatter plot depicting the correlation between the CCL20 and IL-17A levels in the MTX group. Each red asterisk (*) represents a data point for an individual subject within this group. The black line represents the linear regression line fitted to the data, indicating a positive linear trend. The Pearson correlation coefficient (R) for this linear relationship is 0.562. CCL20, chemokine ligand 20.

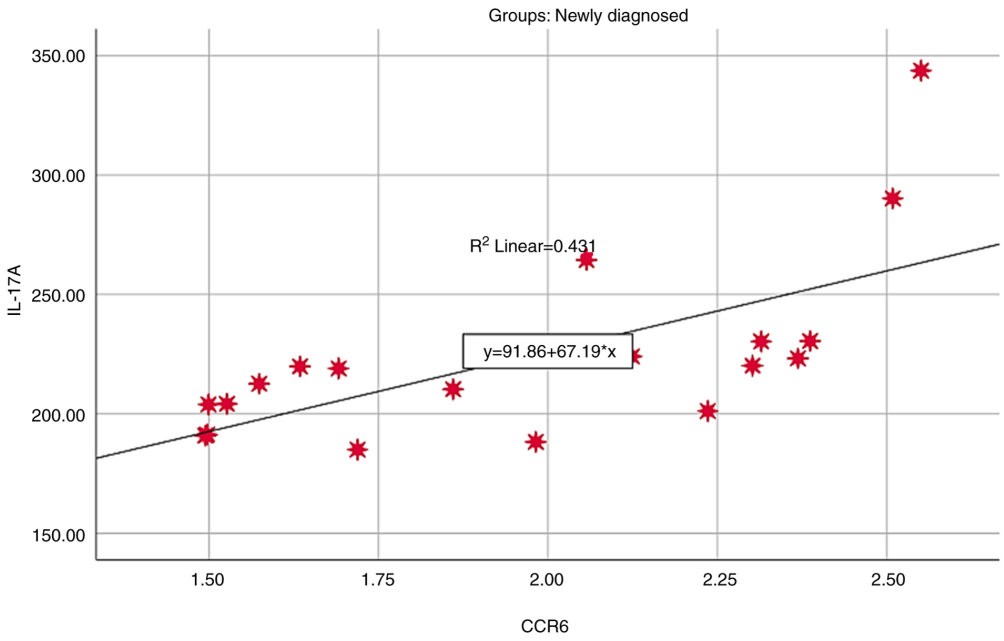

Figure 5. Scatter plot depicting the correlation between the CCR6 and IL-17A levels in the newly diagnosed group. Each red asterisk (*) represents a data point for an individual subject in this group. The black line represents the linear regression line fitted to the data. The coefficient of determination (R^2), indicating the proportion of the variance in IL-17A that is predictable from CCR6, is 0.431 for this linear model. CCR6, C-C chemokine receptor type 6.

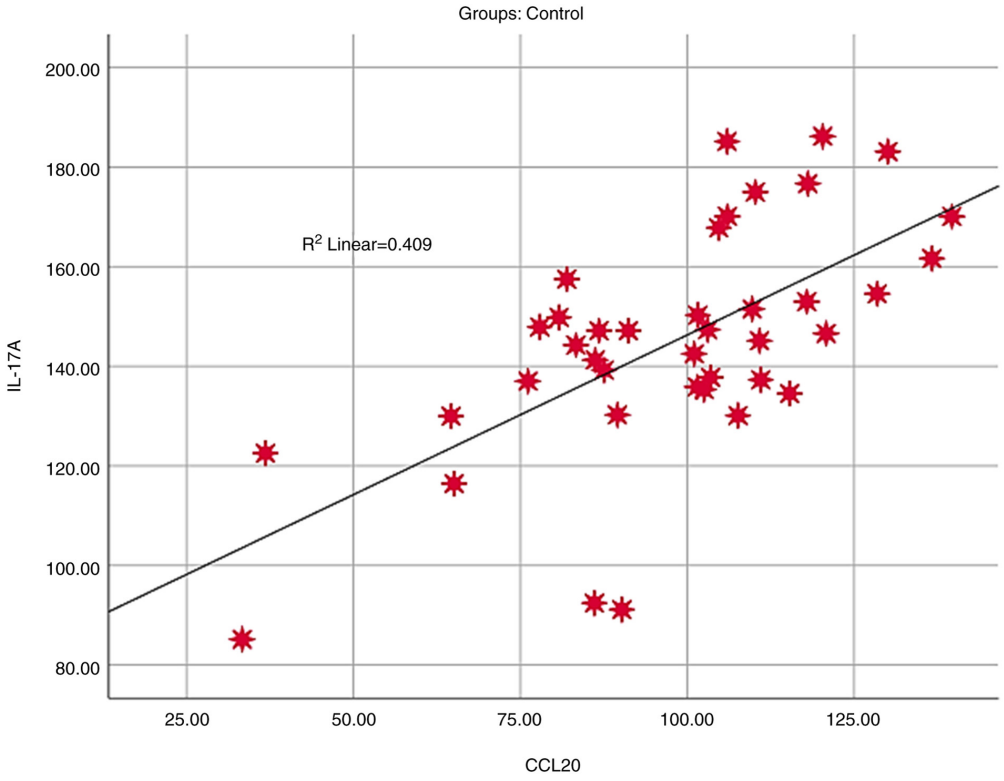

Figure 6. Scatter plot depicting the correlation between the CCL20 and IL-17A levels in the control group. Each red asterisk (*) represents a data point for an individual subject within this group. The black line represents the linear regression line fitted to the data, indicating a positive linear trend. The Pearson correlation coefficient (R) for this linear relationship is 0.409. CCL20, chemokine ligand 20.

this chemokine network has been linked to disease exacerbation. It is known that CCL20 is the only ligand for CCR6. It has been proven that it attracts white blood cells, notably Th17 cells to the inflamed tissues of joints affected by RA. By contrast, the study by Meitei *et al* (17) demonstrated that the CCL20/CCR6 chemical axis contributed to the understanding of the roles in human inflammatory disease, suggesting that CCL20 and CCR6 are present at significantly greater levels in the joints, blood and tissues of patients with RA compared with those noted in healthy controls, indicating a strong association with the severity of RA.

In the present study, a non-significant correlation was demonstrated regarding the link between salivary IL-17A and CCR6 in all groups apart from the newly diagnosed group, where a significant positive correlation was found. This is consistent with the study conducted by Rampersad *et al* (41), who demonstrated a significant correlation between the IL-17, CCR6 and CCL20 levels in patients newly diagnosed with RA. In contrast to these observations, a positive, significant correlation was noted between the levels of IL-17A and CCL20 in each of the control groups, the MTX and the MTX + biotherapy groups; a non-significant correlation was noted in the newly diagnosed RA group. This finding is not in line with a study conducted by Sikorska *et al* (42) who demonstrated a lack of predictive value of IL-17 or CCL20 in patients with long-lasting RA, suggesting that the frequency of Th17 cells and IL-17 levels did not correlate with disease activity in late-stage RA. This was due to the duration and stage of the disease. In the chronic stages of RA or following treatment, the association between IL-17A and CCL20 may be more pronounced due to the existing inflammatory environment. In contrast to these observations, in patients newly diagnosed with RA, inflammatory pathways may not be fully activated or may involve different mechanisms.

The interactions between cytokines and chemokines fluctuate based on the illness stage and treatment setting, with more significant connections noted in particular groups. In patients undergoing MTX or MTX combined with biological therapy, the disease may be better controlled, leading to reduced inflammation levels. As a result, the association between IL-17A and CCR6 may diminish, resulting in non-significant correlations within these groups. In patients newly diagnosed with RA, the inflammatory milieu is characterized by an elevated immune response, resulting in heightened levels of IL-17 and CCL20. This indicates that IL-17A, CCR6 and CCL20 may have unique functions in the pathogenesis of rheumatoid arthritis and responses to treatment (43).

CCL20 levels were significantly lower in patients on MTX or MTX+ biological therapy, which may be attributed to the immunomodulatory role of the therapeutic regimens. Moreover, the MTX + biological group had lower levels of CCL20, which signifies the synergistic effect of MTX + biological therapy (44).

In conclusion, the present study demonstrated that the levels of salivary biomarkers (IL-17A, CCR6 and CCL20) were significantly higher in patients newly diagnosed with RA and that these molecules may serve as biomarkers for the diagnosis of RA. The present study indicated a correlation between CCR6/CCL20 and the emergence of the disease among the Iraqi population. High levels of IL-17A were associated with increased levels of CCR6 and CCL20 in patients with RA, suggesting that this pathway may contribute to the inflammatory process and disease progression in RA. This may highlight potential targets for therapeutic interventions to manage RA more effectively.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YKH was involved in the conception and design of the study, in the literature search, clinical analysis, data analysis, statistical analysis, and in manuscript preparation and manuscript reviewing. BHAG was involved in the conception and design of the study, in data analysis, and in manuscript reparation and manuscript reviewing. Both authors have read and approved the final manuscript. YKH and BHAG confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Prior to data collection, a statement of patient by written informed consent to participate in the study as specified in the Declaration of Helsinki was obtained from each patient. Ethical considerations were obtained from a scientific committee affiliated with the College of Dentistry at Baghdad University (reference no. 863 in November, 2023).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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