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Gene Expressions of CTLA-4 and CCL2 in Patients with Rheumatoid Arthritis

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ABSTRACT

Background: *Rheumatoid arthritis (RA) is an autoimmune disorder in which the immune system erroneously targets the body's own tissues, primarily causing chronic inflammation in the synovial joints (inflammatory arthritis) and potentially affecting other organs and systems (extra-articular manifestations). RA pathogenesis is complicated and it includes genetic predispositions, environmental factors, and dysregulation of the immune system.* ***The aim of this study*** *is to evaluate the correlation between CTLA-4 and CCL2 gene expressions in RA patients.* ***Methods:*** *A total of 120 subjects were involved in a case-control study, including 60 patients with RA and sixty apparently healthy volunteers as a control group. Measurement of the expression levels of CTLA-4 and CCL2 genes was conducted utilizing the real-time PCR methodology.* ***Results:*** *The mean of expression level of the CTLA-4 and CCL2 genes was considerably higher in patients with RA compared with healthy controls (P<0.001), with a significant negative correlation between CTLA-4 and CCL2 gene expressions (R= -0. 248, P=0.04) in patients with RA.* ***Conclusion:*** *This study concluded that CTLA-4 and CCL2 genes have an associated role in the pathogenesis of RA. The balance between the immune-inhibiting CTLA-4 and the inflammation-promoting CCL2 reduces severity of chronic inflammation and tissue damage.*

Keywords: Rheumatoid Arthritis; CTLA-4; CCL2.

Article Information

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder in which the immune system erroneously targets the body's own tissues, primarily causing chronic inflammation in the synovial joints (inflammatory arthritis) and potentially affecting other organs and systems (extra-articular manifestations). This persistent inflammatory process is thought to arise from a complex interplay between genetic predisposition and environmental triggers (1, 2). Cartilage erosion and bone degeneration, which often lead to structural abnormalities, are the end outcomes of rheumatoid arthritis' progressive joint destruction. Patients have a dramatic decline in their lifespan, functional ability, and quality of life as the illness progresses. In addition, the patient's loved ones and caretakers bear a heavy financial and social burden as a result (3).

Rheumatoid nodules, vasculitis, and other extra-articular symptoms may develop from RA's chronic inflammatory state. Systemic consequences can impact several organ and systems, including the respiratory, cardiovascular, neurological, and gastrointestinal systems (4-6). Chronic inflammatory disorder of RA increases the risk of disability and death if not treated (7). Rheumatoid arthritis affects around 1% of the global population, with women having a far greater frequency than males (7, 8). It is believed that about 1% of the Iraqi population suffers with RA (9). The pathogenesis of RA is complex and it includes a significant role of genetic factors in contributes to the susceptibility as well as

the progression of the disease. RA is a complicated autoimmune disease whose heritability is about 60 percent, meaning that it has a strong genetic basis.

Therefore, RA is a genetic disorder that has both non-HLA and HLA genes as part of its genetic architecture, with many loci being found to predispose patients to the disease. Not only do these genetic factors aid in the determination of the probability of developing RA, but also determine the progression and clinical consequences of the disease. The interaction between the genetic predisposition and the environmental factors makes the pathogenesis of RA even more complicated, which is why the detailed understanding of these interactions is needed (10, 11).

The majority of cytotoxic T-lymphocyte associated protein 4 (CTLA-4), also called CD152, is found on regulatory T (Treg) cells and activated T cells. This protein is essential for immunological homeostasis maintenance and has a negative regulatory role in T cells (12). As an immunological counter-regulatory mechanism, CTLA-4 is essential in RA by reducing chronic T-cell activation, a major factor in the development of the illness (13).

Chemokine (C-C) motif ligand 2 (CCL2), often referred to as Monocyte Chemoattractant Protein-1 (MCP-1), is a CC chemokine that induces the movement and recruitment of monocytes, macrophage and T-lymphocyte to site of inflammation (14, 15). CCL2 plays pivotal role in RA by driving the continues influx of inflammatory cells into the joints, perpetuating the inflammatory cycle and tissue damage (16).

The aim of this study is to evaluate the correlation between CTLA-4 and CCL2 in patients with RA.

METHODS

In a case-control research, 120 people were included; among them 60 were patients with RA who sought treatment at Al-Najaf Rehabilitation Centre for Disabled People, Sports Medicine, and Physiotherapy of Al Sadder Medical City, within the period from October 2023 to March 2024. The patients' ages range from twenty to seventy-five, with seven men and fifty-three females. The control group included 55 females and 5 men, ranging in age from 20 to 73 years. They confirmed that there is no known history of RA or other clinical signs, and they did not find any abnormalities.

The study excluded patients with other autoimmune disorders, central nervous system disorders, cardiovascular disorders, immunodeficiency diseases, malignancies, chronic infections, and ages greater than or equal to 75 or less than 20 years. Inclusion criteria included patients diagnosed with RA by a rheumatologist and scoring 6 or higher according to the 2010 ACR/EULAR Criteria. Patients aged 20 to 75 years were also considered.

Total RNA extraction was performed directly after sampling of peripheral blood cells using a complete RNA extraction kit (Solarbio, China), the RNA was extracted from peripheral blood cells as soon as they were sampled. It was determined that the RNA extraction kit was of good quality using the nanodrop technique. The Universal RT-PCR Kit (M-MLV) from Solarbio in China was used for cDNA synthesis.

Measurement of the expression levels of CTLA-4 and CCL2 genes was carried out using the real-time PCR technique in the two separate groups (control and patients). The GAPDH gene served as a housekeeping gene in this study (Table-1). The NCBI web platform was used to appropriately construct

the primers for these two genes. Solarbio Syber Green PCR master mix was used for amplification in the real-time PCR process.

The following components were used to conduct the real-time polymerase chain reaction (RT-PCR): 8 µl of Solarbio Syber Green PCR master mix, 2 µl of forward and reverse primers, 1 µl of cDNA, and 3 µl of nuclease-free water. PCR mixture was prepared for CTLA-4 and CCL2 as target genes and GAPDH as housekeeping or reference gene, which was utilized for the normalization the gene expression of target genes. The amplification protocol was divided into three parts: the hold stage, the thermal amplification cycles (beginning with a 10-minute denaturation at 95 °C, followed by 40 cycles of denaturation, annealing, and elongation at 57 °C, 72 °C, and 44 seconds, respectively), and finally, a melting stage (95 °C, 60 °C, and 15 seconds) to allow for melting curve analysis and product characterization.

All samples' expression levels, measured as the threshold cycle (CT), were normalized using the reference gene GADPH's expression levels. The value of ΔCT for each sample was found by deducting the reference gene's CT value from the target gene's CT value, and the value of ΔΔCT was defined as the variance of the experimental group's ΔCT and the control group's ΔCT. Afterwards, the amounts of relative expression for the Beclin-1 and Atg5 genes were calculated using the $2^{-\Delta\Delta CT}$ technique.

Table 1: The Primers and their Sequences

Primer	Orientation	The sequence	length
CTLA-4	Forward	TGGCTTGCCTTGGATTTCAGC	173bp
	Reverse	ACACACAAAGCTGGCGATGC	
CCL-2	Forward	CCGAGAGGCTGAGACTAAC	241bp
	Reverse	CTTGCTGCTGGTGATTCTTC	
GAPDH	Forward	CTCCTCACAGTTGCCATGTA	98bp
	Reverse	GTTGAGCACAGGGTACTTTATTG	

STATISTICAL ANALYSIS

Data from each study groups were inputted and analysed utilising the Statistical Package for the Social Sciences (SPSS) (version 27, Inc., Chicago, USA). Descriptive data were reported as mean and SD, frequencies, and proportions. The study used several statistical tests, such as Pearson's Chi-square, Fisher's exact tests, Spearman's rank correlation coefficient, ANOVA test, and independent t-test was utilized to assess correlations among biomarkers, laboratory parameters. Moreover, significance level of <0.05 was deemed indicative of a meaningful difference or correlation.

RESULTS

The present study included 60 patients diagnosed with RA and 60 healthy controls. Table (2) presents the demographic characteristics of both patients and the control persons. The mean age of patients was 45.93 year, whereas the

mean age of control individuals was 45.78 year. There was no significant difference in mean age was observed between patients and control (P= 0.95). The patients group comprised 7 (11.7%) males and 53 (88.3%) females, whereas the control group included 5 (8.3%) males and 55 (91.7%) females. Non-significant difference was seen in the distribution of males and females between the patients group and control group (P= 0.54). Furthermore, the mean and standard deviation of the BMI of patients with RA were 29.47 ± 5.53 kg/m2 and control individuals were 25.95±3.56 kg/m2 with a significant variations (P=0.001). The comparison of CTLA-4 and CCL2 gene expression was conducted between patients with RA and control participants. The results is shown in table (3).The gene expression of CTLA-4 and CCL2 genes were significantly increased in individuals with RA compared to healthy controls (P<0.001).

A correlation of CTLA-4 gene expression with demographic parameters, BMI, and treatment history aspects in RA patients has been performed, with results presented in table (4). The present findings indicate that CTLA-4 gene expression exhibited no significant negative correlation with, age, family history, duration of the disease, regularity of treatment intake [(R= -0.011, P= 0.93), (R= -0.062, P= 0.64), (R= -0.043, P= 0.74), and (R= -0.029, P= 0.33), respectively].

Nonetheless, there was a non-significant positive correlation of CTLA-4 gene expression with sex, BMI and treatment response [(R= 0.158, P= 0.23), (R= 0.009, P= 0.94) and (R= 0.049, P= 0.71), respectively].

A correlation between the expression of CCL2 gene and the demographic characteristics, BMI, and treatment history features in RA patients has been performed, with the results presented in table (5). The current data indicate that CCL2 gene expression exhibited a non-significant negative correlation with age, and sex, family history [(R= -0.097, P= 0.46) and (R= -0.050, P= 0.71), respectively].

Nonetheless, there was a non-significant positive correlation between CCL2 gene expression and BMI, duration of the disease and treatment response [(R= 0.051, P= 0.69), (R= 0.070, P= 0.59) and (R= 0.208, P= 0.11), respectively]. The expression of CCL2 gene exhibited a significant positive correlation with regularity of treatment (R= 0.268, P= 0.04), and highly significant negative correlation with family history (R= -0.327, P= 0.01).

The relationship between the expression of CTLA-4 gene and hematological markers in patients with RA is presented in table (6). The current findings indicate a non-significant positive correlation of CTLA-4 gene expression with HB level (R=0.138, P< 0.29) and platelets count (R= 0.048, P= 0.71), as well as CCL2 gene expression with WBC count (R=0.035, P< 0.79), and platelets count (R= 0.031, P= 0.082). This study demonstrates a non-significant negative association between CTLA-4 gene expression and WBC count (R= -0.192, P= 0.14), as well as between CCL2 and HB level (R= -0.006, P= 0.096).

Table (7) shows the correlation between the expression of the CTLA-4 and CCL2 genes. The present study demonstrates a significant negative correlation between CTLA-4 and CCL2 gene expressions (R= -0.248, P=0.04).

Table (2): Demographic and Anthropometric Characteristics of the RA Patients and Controls.

Characteristics	Patients *n*= 60	Controls *n*= 60	*P value*
Age (years)			
Mean±SD	45.93 ± 12.38	45.78 ± 11.87	0.95 † NS
Range	23–75 years	21–70 years	
Sex			
Male, *n* (%)	7 (11.7%)	5 (8.3%)	0.54 † NS
Female, *n* (%)	53 (88.3%)	55 (91.7%)	
Body mass index (BMI) kg/m2			
Mean ± SD **(kg/m^2)**	29.47 ± 5.53	25.95 ± 3.56	< 0.001 † HS
Range **(kg/m^2)**	19 – 45	19 – 45	

***SD**: standard deviation**; n**: number of cases; **NS**: not significant at P>0.05; **¥**: Chi-square test; †: independent samples t-test*.

Table (3): CTLA-4 and CCL2 Gene Expressions in the RA Patients and Controls.

Genes	Gene expression (Mean ± SD)		Fold	*P value*
	Patients *n* = 60	Controls *n* = 60		
CTLA-4	1.89 ± 0.74	0.99 ±0.21	1.9	<0.001 † HS
CCL2	2.21 ± 1.00	1.06 ±0.22	2.1	<0.001† HS

***SD**: standard deviation**; n**: number of cases; **HS**: highly significant at P<0.05; †: independent samples t-test.*

Table (4): Correlations of the Demographic Characteristics, BMI and Features of Treatment History with CTLA-4 Gene Expression in the RA Patients.

Characteristics	CTLA-4	
	Correlation Coefficient (r)	P-value
Age	-0.011	0.93
Sex	0.158	0.23
BMI	0.009	0.94

Characteristics	CTLA-4	
	Correlation Coefficient (r)	P-value
Family History	-0.062	0.64
Duration of Disease	-0.043	0.74
Treatment Intake	-0.029	0.83
Treatment Response	0.049	0.71

R: *correlation coefficient;* ***P:*** *p value.*

Table (5): Correlations of the Demographic Characteristics, BMI and Features of Treatment History with CCL2 Gene Expression in the RA Patients.

Characteristics	CCL2	
	Correlation Coefficient (r)	P-value
Age	-0.097	0.46
Sex	-0.050	0.71
BMI	0.051	0.69
Family History	-0.327	0.01
Duration of Disease	0.070	0.59
Treatment Intake	0.268	0.04
Treatment Response	0.208	0.11

R: *correlation coefficient;* ***P:*** *p value.*

Table (6): Correlation of CTLA-4 and CCL2 Gene Expressions with the Hematological Parameters of the Patients with RA.

Parameters	CTLA-4		CCL2	
	R	***P***	***R***	***P***
HB level	0.138	0.29	-0.006	0.96
WBC count	-0.192	0.14	0.035	0.79
Platelets count	0.048	0.71	0.031	0.82

R**:** correlation coefficient; ***P*****:** p value.

Table (7): Correlation between CTLA-4 and CCL2 Gene Expressions.

Parameters	CTLA-4		CCL2	
	R	***P***	***R***	***P***
CTLA-4	1	-	-0. 248	0.04
CCL2	-0. 248	0.04	1	-

R: *correlation coefficient;* ***P:*** *p value.*

DISCUSSION

In the current study, the average age of the patients with RA was 45.93 ± 12.38 years, which is predominantly consistent with the reports by Chiad *et al.* (17), Shrivastava *et al*. (18) and Albarzinji *et al.* (19). These studies found average ages of 46.16, ±1.24 years, 46.71, ± 7.12 years, 47.4, ± 10.6 years, and 45.67 11.2 years, respectively. Although persons can develop RA at any age, the risk is high in people, who are at their forties and fifties (20). It is unknown how RA develops in the elderly, but age weakens the immune system, making them more susceptible. T cells are primarily influenced by "immunosenescence"; the immune system is considerably affected by aging. Ageing is linked to infections, cancer, and autoimmune diseases like RA (21).

According to the findings of the current research, RA patients are predominantly females, where 88.3% of patients were female, whereas 11.7% were male. This finding aligns with previous researches done in Iraq by Jwad *et al*. (22), as well as study in Spain by Silva-Fernández *et al*. (23) and in Morocco by Lamkhanat *et al*. (24). RA is more common in women than males, suggesting a sex-based susceptibility discrepancy due to sex hormone variations throughout puberty, pregnancy, breastfeeding, and menopause. These hormonal alterations, especially estrogen levels, boost antibody production and reduce pro-inflammatory responses related to autoimmune dysregulation (25-27).

The present study shows that the mean BMI in RA patients was 29.47 kg/m², closely aligning with the finding conducted by Roman *et al*. (28) and Abubaker & Sinjari (29), which documented mean BMIs of 29.6 kg/m² and 28.72 kg/m², respectively, in RA patients. The mean for controls was 25.95 kg/m, there was a significant vibration seen between patients and controls (p= 0.001). Despite several postulated theories, obesity's role in RA etiology is unclear. Weight increase and adipose tissue buildup may explain the obesity-chronic inflammation link. Adipose growth may increase adipocytokines and pro-inflammatory cytokines, worsening inflammation. Many fat people also have vitamin D insufficiency. Evidence shows vitamin D supplementation may reduce RA risk (30-32).

This study demonstrated that the gene expression level of CTLA-4 in patients with RA was significantly higher compared to controls, with a statistically significant difference (P< 0.001). The mean of gene expression level of the CTLA-4 gene was considerably higher in patients with RA (1.89 ± 0.74) compared with healthy controls (0.99 ± 0.21), with a significant p value (p<0.001). This findings lead into the expression of CTLA-4 gene in RA patients increased 1.9 fold compared with healthy control. This result matches with the findings

of Abdelnaby *et al.* (33), which demonstrated that CTLA-4 gene expression was higher in RA patients compared to healthy controls, exhibiting a statistically significant difference (P < 0.005), with higher median (inter-quartile range) [1.353(0.73) VS. 1]. Also there was a study conducted by Aihaiti *et al.* (34) demonstrated that the expression of the CTLA-4 gene in synovial tissue of RA patients was overexpressed compare to healthy individuals.

In addition, the finding of the present study compatible with research conducted by Liu *et al* (35) when they found increased expression of CTLA-4 in peripheral blood T-Cell with a mean +/- SD of 1. 89 +/- 1.92 and this expression exert down-regulator effect on pro-inflammatory cytokine. Furthermore, this outcome agrees with the previous research by Gouda *et al.* (36), which indicated that CTLA-4 expression was 1.59 folds greater in RA patients than in healthy controls, and 1.40 folds greater in patients with other autoimmune disease like vitiligo.

In addition to the expression of CTLA-4 gene in RA patients, Cao *et al*. (37) found that increased serum CTLA-4 (sCTLA-4) in RA patients was correlated significantly with disease activity score (DAS-28).

The gene expression level of CCL2 in RA patients was higher than the healthy controls with a highly significant statistically difference (P < 0. 001) with a mean of 2.22 ± 1.00 in RA patients and 1.06 ± 0.22 in controls. This outcome agrees with a previous study by Zhang *et al.* (38), who demonstrated that the CCL2 gene expression was 1.91 -fold higher in patients with RA compared to healthy subjects (P= 0.05). In addition, they found the expression of CCL2 in synovial tissue of RA patients was 7.18 folds higher (p< 0.05) compared with control. In addition to that, serum CCL2 was higher in RA patients compared with control.

Also, this result aligns with the findings of Abdelnaby *et al.* (33), which demonstrated that CCL2 gene expression was significantly higher over-expressed in RA patients in compared with control. Furthermore, these significant differences of CTLA-4 and CCL2 gene expressions indicate a role of these two genes in the severity and progression of RA.

The higher gene expression levels of CTLA-4 in RA patients comparative to healthy peoples can be explained as a counter-regulatory attempt by the immune system to suppress chronic T-cell activation that fuels the RA disease and cause its progression (39, 40). Whereas, the explanation of increased expression of CCL2 gene in RA patients is due to the role of CCL2 as a chemotactic factor that driving the continuous influx of inflammatory cells into the joints. The upregulation of both genes creates a synergistic cycle of inflammation and autoimmunity that disrupt immune hemostasis, perpetuates chronic inflammation, joint damage and drives disease progression. The inflammatory response is perpetuated by a dual mechanism: CTLA-4 dysfunction causes uncontrolled T cell activation, while CCL2 facilitates the recruitment of additional immune cells (41, 42).

The present study identified a non-significant positive correlation of CTLA-4 gene expression with sex, BMI and treatment response, in addition to a non-significant negative correlation with age, family history, duration of disease and regularity of treatment intake. The findings of non-significant correlations of CTLA-4 gene expression with sex, family history, and

treatment intake in RA patients are consistent with prior research by Abdelnaby *et al.* (33). We found no comparative data for other parameters, as no previous studies had evaluated the relationship of BMI, duration of disease and regularity of treatment intake with CTLA-4 gene expression.

This study identifies a non-significant positive correlation of CCL2 gene expression with BMI, duration of disease, regularity of treatment and treatment response. The current study revealed that CCL2 gene expression exhibited a non-significant negative correlation with age, sex in patients with RA. The expression of CCL2 gene exhibited highly significant negative correlation with family history (R= -0.327, P= 0.01) and significant positive correlation with regularity of treatment intake (R= 0.268, P= 0.04).

The findings indicate a non-significant correlation of CCL2 gene expression with sex in RA patients, that consistent with prior research by Abdelnaby *et al.* (33) which also demonstrated a non-significant correlation between CCL2 gene expression and these demographic and clinical characteristics.

The duration of disease may affect CCL2 expression, the chronic nature of RA suggests that extended duration of disease could potentially result in the maintenance of elevated CCL2 expression as a result of continues inflammatory process in RA, this result conducted by Zhang *et al.* (38) and aligns with the results of the current study when found a positive correlation between CCL2 expression and disease duration.

Based on the correlation of CCL2 gene expression with BMI in RA patients, and considering our expertise in the subject, no comparative results exist, as no previous studies have evaluated the correlation of CCL2 gene expression with BMI, and hypertension in RA patients.

The pivotal role of CCL2 in inflammatory processes notwithstanding its systemic levels may not adequately reflect its role in specific diseases or conditions. The redundancy and complexity of the chemokine network along with the localized nature of its action likely contribute to the observed absence of significant correlations, Furthermore, functional compensation by alternative chemokines and their cognate receptors is likely to obscure any direct relationship between CCL2 levels and metabolic or disease-related parameters (43-46).

This study demonstrated a significant relationship of CTLA-4 gene expression with hematological parameters, including hemoglobin levels, white blood cell count, and platelets count in patients with RA. The findings demonstrate a non-significant positive correlation of CTLA-4 gene expression with hemoglobin level ((R=0.138, P< 0.29) and platelets count (R= 0.048, P= 0.71), alongside a non-significant negative correlations of CTLA-4 gene expression with WBC count (R= -0.192, P= 0.14).

According to our knowledge about the subject, there were no results available for comparison with these findings, as no previous studies have evaluated the link of CTLA-4 gene expression levels with hemoglobin levels, WBCs count and platelets count in patients with RA. Where, the CTLA-4 role is more closely related to immune regulation rather than hematological parameters. Therefore, its role does not extend to directly affecting hematological parameters such as hemoglobin and platelet counts. Genetic studies have not found a consistent association between CTLA-4

polymorphisms and hematological parameters in RA, further supporting the notion that CTLA-4's influence is more immunological than hematological (47, 48)

The present study identified a non-significant association between CCL2 gene expression and hematological parameters in patients with RA. The current study reveals non-significant positive associations of CCL2 gene expression with white blood cells count, and platelets count (R=0.035, P< 0.79 and R= 0.031, P= 0.082, respectively). Alongside, this study demonstrates a non-significant negative association between CCL2 gene expression and HB level (R= -0.006, P= 0.096).

As far as our knowledge about the subject, there were no prior studies available for comparison regarding the relationship between the gene expression of CCL2 and the hematological parameters. Although CCL2 plays a pivotal role in the inflammatory processes of RA, particularly in monocyte recruitment, it does not directly modulate hematological parameters. These parameters are governed by an intricate interplay of various cytokines, disease activity, and systemic inflammation, which are not exclusively dependent on CCL2 levels. This underscores the multifaceted nature of RA pathogenesis and emphasizes the necessity of a holistic approach to elucidate the disease's underlying mechanisms (49-51).

The current research findings indicate a significant negative correlation between the expression of CTLA-4 and CCL2 genes (R= -0.248, P= 0.04). The expression of CTLA-4 and CCL2 genes shows a significant negative correlation which can be attributed to their opposing roles in immune regulation and inflammation. The CTLA-4 gene is a regulatory gene, and its role in RA reflects a counter-regulatory attempt by the immune system to inhibit chronic T-cell activation, which is a hallmark of RA, CTLA-4 acting as a checkpoint that dampens immune responses, whereas the CCL2 gene encodes a chemokine that drives the recruitment of inflammatory cells into the joint and perpetuates the inflammatory cycle and joint destruction. This inverse relationship is evident in various pathological contexts, where the balance between immune activation and suppression is crucial (40-42).

The current study contradictory with modern findings by Abdelnaby *et al.* (33), which demonstrated that there is no significant correlation between CTLA-4 and CCL2 expression among RA (R= 0.087, P= 0.551).

LIMITATIONS

The study acknowledges that the sample size was relatively small. Future research should involve a larger group of participants to validate the findings and ensure that the results are generalizable to a broader population. Further studies are needed to assess CTLA-4 and CCL2 gene expressions in the synovial fluid of patients with RA. Additional multi-center studies are needed to be conducted. The research was conducted at a single medical center, which may introduce biases related to the specific patient population and clinical practices at that institution. Multi-center studies would provide a more comprehensive understanding of the findings across different settings. More future researches are required to involve the excluded RA patients. The study excluded patients with various conditions, such as malignancy and cardiovascular diseases. This limits the generalizability of the findings, as the results

may not apply to patients with these comorbidities.

CONCLUSIONS

This study concluded that CTLA-4 and CCL2 genes have an associated role in the pathogenesis of RA, because the findings revealed that the gene expression levels of CTLA-4 and CCL2 in RA patients are significantly higher than in healthy control group. The balance between the immune-inhibiting CTLA-4 and the inflammation-promoting CCL2 reduces severity of chronic inflammation and tissue damage, because the findings highlight a strong negative correlation between the gene expression levels of CTLA-4 and CCL2.

Ethical consideration

Before the start of the research project, this study was approved through the ethical committee of the College of Medicine, University of Kufa with the number (009647801241456). Informed agreement was obtained from each patient, and agreement was obtained from the Unit of Rheumatology at Al Sadder Medical City in Al-Najaf city, and the patient's consent was also obtained to conduct a questionnaire and collect a blood sample.

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