

The role of CCR6 rs 3093024 in Rheumatoid Arthritis patients in Iraq

Aya Maher Kamil¹, Mouhammad Mahmoud Farhan AL-Halbosiy², Abd alameer Nasir Ghalob AL-Rekabi³

^{1,2,3}AL-Mustansiriyah University, College of science, Department of Biology, Iraq

Email: aya980500@gmail.com

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Abstract

Rheumatoid arthritis (RA) is a chronic auto immune and inflammatory disease of obscure etiology. But the emergence of disease is mostly influenced by genetic, immunological, and environmental factors. This study was investigated the polymorphisms of SNP (rs3093024) of CCR6 gene and its association with susceptibility to RA, serum level of the chemokine CCR6 also were investigated to determine its association with RA disease. Accordingly, a case-control analysis was carried out on 60 patients with RA and 30 healthy control. The ELISA result indicated that the CCR6 mean was increased highly significant in the serum of RA patient than the serum level of control (299.19 ± 17.12 versus 149.52 ± 5.36 pg/ml, $p=0.0001^{**}$). For CCR6 rs3093024 A>G logistic regression analysis result revealed that both of the alleles (A and G) and genotypes (AA, AG and GG) was not associated with susceptibility to RA in the present sample of Iraqi patients.

Keywords: polymorphisms, Rheumatoid arthritis (RA), Iraqi patients.

1. INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that affects numerous joints on both sides of the body. It is characterized by tendon inflammation (tenosynovitis) that causes both bone erosion and cartilage destruction (Lin et al,2020).). Although genetic, environmental, and serological aspects have been found to contribute to the onset and course of the disease, the cause of rheumatoid arthritis (RA) is unknown. Genetics account for between 50 and 60 percent and therefore , genetic factors have a significant influence on susceptibility to RA (Verheul et al ,2015). RA was 2- to 3-fold more frequent in females than in males (Lin et al, 2017; Kato et al, 2017). Chemotactic cytokines known as chemokines that control immune cell movement in a variety of normal and pathological processes. They are essential for maintaining homeostasis, producing cellular and humoral immune responses, and contributing to pathologic immunity in a number of diseases (Ce , 2018). The IL-17-producing Th17 cells are a subpopulation of CD4+ T helper (Th) cells that express CCR6 (CC chemokine receptor 6), which is coupled to its ligand (CCL20), causing these cells to homing to inflamed joints. Th17 cells play a significant role in the pathophysiology of RA by generating IL-17, a well-known inflammatory cytokine in most autoimmune disease (Mohammadi Kebar et al ,2018). A C-C chemokine receptor type 6 protein with a length of 374 amino acids is encoded by the CCR6 gene, which is found on chromosome 6 (6q27) (Figure 1-4). It is one of the genes with a potential role in the development of RA. Because it encodes a chemokine receptor that is crucial for B cell differentiation and migration during inflammatory and immune responses, this gene is very relevant to autoimmune disease (Salazar-Gonzalez et al, 2006). The ligand of this receptor is the macrophage inflammatory protein 3 alpha (MIP-3 alpha) (Serrano et al,2013). The chemokine (C-C motif) receptor 6 gene (CCR6) has recently been discovered as a susceptibility locus for RA by three independent GWAS investigations (2 on Asian and 1 on European populations) (Jiang et al , 2014 ;Stahl et al ,2010).). The CCR6 gene was found to have a considerable connection with RA in the Japanese population, and the findings suggested that the gene may play a significant pathogenic role in the illness. However, additional investigations with African American groups revealed contradictory results. Due to this discrepancy, it is important to evaluate the function of CCR6 polymorphisms in RA (Huges et al,2010;Perkins et al ,2012).

2. Material and method

2.1 Subjects

In this study , the blood samples were collected from 60 patients who admitted the department of chronic arthritis diseases Baghdad teaching hospital / medical city / Baghdad / Iraq and 30 healthy control from Iraqi population that were selected

randomly with no family history of RA or other autoimmune disease. A physical examination and medical history were checked in all patients. The excluded patient with hepatic disease, malignant illness, cardiac failure, renal failure, and psoriasis. Nearly five milliliters of blood were collected from each patient and separated into two parts. The first blood part was collected in a tubes containing EDTA (2 ml) to use for genetic analysis. While the second part collected in a gel tube to use in ELISA technique and for other laboratory analysis. An informed consent form was signed from each subject included in the study, and the study was approved by the Ethical committee of Department of life science, Iraq.

2.2. DNA extraction and genotyping

The DNA extraction from the blood of patients and control subjects by Easy Pure Blood Genomic DNA kit. Then agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA (Russell and Sambrook, 2001). In order to detect the genetic variation in the two studied groups and its different genotypes in CCR6 gene for SNP (rs3093024 A>G), HRM real-time polymerase chain reaction (HRM RT-PCR) were used. Primers used in this study were designed according to their reference sequence in the National Center for Biotechnology Information's database (NCBI), the primers that were used forward CTCCTCTGTGCTCCCTCCT and reverse CAGAGAGCCTACGTGTGAC , the two primers were used in Real Time PCR reaction mixture. The thermal profile of HRM genotyping : Enzyme activation at 94°C for 60 sec which was followed by 40 cycle of , Denaturation at 94°C for 5 sec , Annealing at 58 °C for 15 sec ,Extension at 72 °C for 20 sec, HRM at temperature between 65-95 °C for 0.2 sec for 1 degree.

2.3 Measurement the serum level of the chemokine

The level of CCR6 in serum of RA patient and control was measured by using commercial ELISA kit from My Biosource company (USA) according to the manufacturer's procedure.

2.2. Statistical analysis

To identify the impact of various factors on study parameters, the Statistical Analysis System- SAS (2018) program was utilized. Chi-square test was performed to compare percentage (0.05 and 0.01 likelihood) in a significant way. Estimated correlation coefficient between the study's variables.

Both the odds ratio, which was evaluated using a unique 2 formula by Abramson in 2011 (Rodriguez et al. in 2009), and the P values computed with Fisher's exact test were statistically evaluated using the WINPEPI computer program (version 11.63). The chi squared test was used to examine the Hardy-Weinberg equilibrium utilizing studies from the OEGE (Online Encyclopedia for Genetic Epidemiology) database (Barret et al,2005).

3.Result

3.1 Clinical and demographic characteristics of RA patients and healthy controls.

The clinical and demographic characteristics of RA patients and healthy controls are listed in (Table1). Mean age of RA patients was highly significant increased compared with controls (46.23 ± 1.46 vs. 38.04 ± 2.07 year; $p = 0.001^{**}$). Females were more commonly occurring than males in RA patients (83.33 vs 16.67%) and control (74.07 vs 25.93%). The mean of BMI for patients were highly significant than mean of BMI of healthy control (28.44 ± 0.63 vs. 25.02 ± 0.49 K.g/m², $P = 0.001^{**}$). The ESR mean in patient was highly significant increased than healthy control (41.73 ± 3.19 versus 9.2 ± 0.65 mm/hour, $P = 0.0001^{**}$)(Table 1).

Table 1 Clinical and demographic characteristics of RA patients and healthy controls

variable	patient	control	P value
Age in years Mean \pm SE	46.23 ± 1.46	37.433 ± 1.92	0.001^{**}
Gender			
Male	16.67%	25.93%	0.383
Female	83.33%	74.07%	
BMI Mean \pm SE	28.44 ± 0.63	25.02 ± 0.49	0.001^{**}
ESR Mean \pm SE	41.733 ± 3.19	9.2 ± 0.65	0.0001^{**}

3.2 The serum level of CCR6 in Rheumatoid Arthritis patient.

The mean of CCR6 level in the serum of RA patient was highly significant than the serum level of control (299.19 ± 17.12 vs 149.52 ± 5.36 pg/ml, $p = 0.0001^{**}$) (Figure 1).

CCR6-level

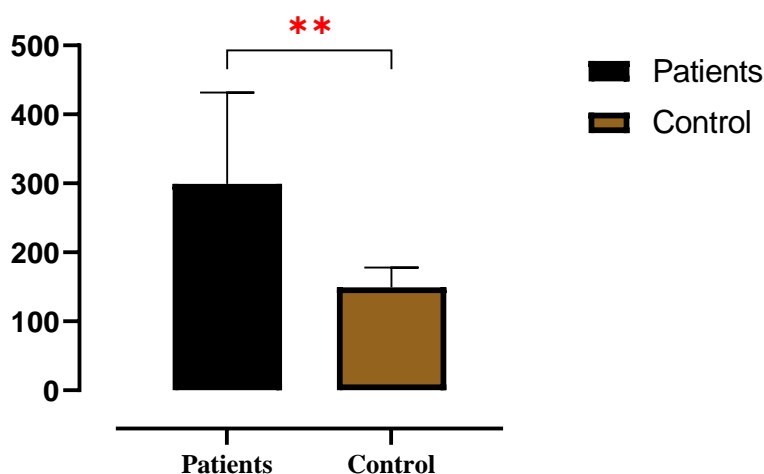


Figure (1) Serum level of CCR6 in Rheumatoid arthritis patient and control.

3.2. Association of CCR6 rs3093024 with Rheumatoid Arthritis.

The SNPs detection was achieved by using HRM real-time PCR. Three genotypes were identified; AA, AG and GG, which were correspondent to two alleles; A and G.

Inspecting the rs3093024 of CCR6 gene genotyping polymorphism revealed that the AA genotype was present in 51.67% of RA patient versus 60% in control [P=0.438, OR=0.71, 95% CI(0.26—1.89)]. AG genotype was present in 26.67% of RA patient versus 26.67% of control [P=0.9, OR=1.00, 95% CI(0.37—2.82)]. GG genotype was present in 21.67% of RA patient versus 13.33% of control [P=0.329, OR=1.80, 95% CI(0.54-6.94)]. The frequency of A allele 65% in RA patient versus 73.33% in control [P=0.275, OR=0.68, 95% CI(0.33-1.33)]. The frequency of G allele in RA patient 35% versus 26.67% in control [P=0.275, OR=1.48, 95% CI(0.75---2.99)]. The results shows that there was a non significant differences between patient and control these revealed that rs3093024 of CCR6 gene has no association with RA disease among Iraqi patient (Table 2)

Table (2) Comparison of the Genotype and Allele Frequencies of CCR6 gene polymorphism (rs3093024 A>G) between Patients Group and Control group

Genotype/ Allele	RA group N=60	%	C group N=30	%	P value	OR	95% CI
AA	31	51.67%	18	60%	0.438	0.71	(0.26-1.89)
AG	16	26.67%	8	26.67%	0.9	1.00	(0.37-2.82)
GG	13	21.67%	4	13.33%	0.329	1.80	(0.54-6.94)
A	78	65%	44	73.33%	0.275	0.68	(0.33-1.33)
G	42	35%	16	26.67%	0.275	1.48	(0.75-2.99)

The distribution of genotype frequencies in control subjects was in a consistency with HWE, and no significant difference was saw between observed and expected genotype frequencies of the SNP rs3093024 (p = 0.081). However, among RA patients, there was a significant deviation from HWE (p = 0.001) (Table 3).

Table (3) Numbers and Percentage Frequencies (observed and expected) of CCR6 gene (rs3093024) Genotypes and their Hardy-Weinberg Equilibrium (HWE) in RA Patients and Controls.

Genotype	Rheumatoid arthritis Patient N=60		Control N=30	
	Observed		Expected	
	No	%	No	%
AA	31	51.67%	25.35	42.25%
AG	16	26.67%	27.3	45.5%
GG	13	21.67%	7.35	12.25%
HWE Analysis	P=0.001		P=0.081	

4. Discussion

The result of this study revealed that CCR6 level in the serum of RA patients was highly significant increased than control. One of a group of receptors that reacts to environmental signals and directs the migration of inflammatory cells to the site of inflammation is the chemokine receptor 6 (CCR6). It has been shown to be expressed on several cell types important to the development of inflammatory arthritis (Acosta-Rodriguez et al,2007; Manel et al,2008). The discovery of higher CCL20 levels in the joints of RA patients as compared to individuals with osteoarthritis further supports the involvement of CCR6 in RA (Hirota et al, 2007). CCR6+ Th cells have been seen in synovial fluid, inflammatory synovial tissue, and peripheral blood in RA patients. A chronic arthritis may develop from an early inflammation that is derived by CCR6+ Th cells (Paulissen et al, 2015). Matsui et al (2001) showed the revelation of CCR6 in infiltrating mononuclear cells in the cellular mass and around the vessels of RA synovial tissue. Lee and Körner (2014) mentioned that CCR6+ inflammatory cells could be attracted by CCL20 that secreted by FLS and osteoblasts, to the synovial joints, and starting immune-mediated destruction. Lee and co-workers (2017) revealed a probable essential role for CCR6 in the immunopathogenesis of SLE, They were also informed about the importance of CCL20, a ligand that partners with CCR6 in the recruitment of CCR6+ B cells to effector locations.

According to research by Furue and co-workers (2020), the CCL20/CCR6 axis is elevated in human psoriasis lesional skin and in experimental psoriasiform dermatitis. As a result, blocking the CCL20/CCR6 axis using a particular antibody or pseudoagonist reduces the severity of the condition.

In this study the association of CCR6 rs3093024 with RA was studied , revealing that the three genotypes AA ,AG,GG and the alleles A and G was not associated with RA disease in Iraqi population.

According to a study by Teng et al (2012) on Asian populations, CCR6 polymorphism was protective factor in men but risk factor in women. In studies made by Kochi et al (2010) and Stahl et al (2010) mentioned that the A allele of CCR6 gene polymorphism was shown to increase the risk of RA.

Akhatar et al (2020) showed that the CCR6 rs3093024 has a significant association with RA and the A-allele is a risk-factor for Pakistani population. All of these above studies disagree with the current study. Another study conducted on the Korean population revealed a significant correlation between this SNP and RA (Freudenberg et al , 2011). Ochoa and co-workers (2015) showed a significant correlation between SNP rs3093024 with SSc auto immune disease patients comparing with control.

Chang and co-workers (2012) studies on Taiwanese population showed a significant association between the genotype of CCR6 (rs3093024) and the potentially for RA which was inconsistent with the current study, while they revealed that rs3093024 was not associated with susceptibility to AS auto immune disease and that agree with our results. Yi et al (2011) revealed that there was no association of CCR6 (rs3093024) with susceptibility to Behçet's disease in Chinese population.

The difference between the prior study on different population and this study could be due to ethnicity difference among different population caused by genetic variation and differences in environmental influences.

Many studies have shown a strong correlation between other CCR6 variations and RA, however only a small number of studies have found a connection between CCR6 rs3093024 and RA. Although the deep intronic SNPs are not known to have a role in disease pathogenesis, their pathogenicity mechanism should be investigated given that they are frequently linked to risk factors

for a variety of illnesses, including autoimmune diseases (Akhtar et al, 2020).

5. Conclusions

In conclusion, the serum level of CCR6 was increased significantly in RA patients comparing with control. Single nucleotide polymorphism rs3093024 of CCR6 gene revealed that none of the alleles or genotypes were associated with susceptibility to RA disease, however more studies are needed to assure the role of CCR6 in Rheumatoid Arthritis in Iraqi population.

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