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Assessment of the *−174G/C* (rs1800795) and *−572G/C* (rs1800796) *Interleukin 6* Gene Polymorphisms in Egyptian Patients with Rheumatoid Arthritis

Khalda Amr[1], Rehab El-Awady[2*], Hala Raslan[1]

[1]National Research Center, Internal Medicine, Cairo, Egypt; [2]Al-Azhar University Faculty of Pharmacy, Biochemistry, Cairo, Egypt

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Keywords: Rheumatoid arthritis; Interleukin 6; *IL6–174G/C, IL6–572G/C*; gene polymorphism.

***Correspondence:** Rehab El-Awady. Al-Azhar University Faculty of Pharmacy, Biochemistry, Cairo, Egypt. E-mail: r.awady@yahoo.com

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Abstract

AIM: This study aimed to investigate genotype and allele frequencies of −174 (rs1800795) and *−572* (rs1800796) *IL-6* promoter gene polymorphisms in Egyptian patients with rheumatoid arthritis (RA) in comparison to control group.

METHODS: The study was conducted on 198 Egyptian subjects (99 RA patients and 99 healthy control).The promoter region of *the IL-6* gene was amplified by PCR using DNAs from patients and the controls, and their PCR products were digested by suitable enzymes.

RESULTS: No statistical differences were found in *−572G/C* genotype (P = 0.177) or allele (P = 0.147) frequencies between RA patients and controls. Significant differences were observed in *−174G/C* genotype (P < 0.001) and allele (P < 0.001) frequencies between RA patients and controls.

CONCLUSION: A significant association of *IL-6 −174G/C* gene polymorphism and RA in Egyptian population was found with significantly higher frequencies of *GC* and *CC* genotypes and *C* allele in RA patients compared to controls. No association was found between *IL-6 −572G/C* gene polymorphism and RA.

Introduction

Rheumatoid arthritis (RA) is a multisystemic autoimmune inflammatory disease which has the prevalence of approximately 1% worldwide [1]. The interaction of genetic and environmental factors results in a cascade of immune reactions, which contributed to the pathogenesis of RA. The common manifestations of RA include synovitis, joint damage, and structural cartilage and bone damage. Also, uncontrolled active RA causes disability, diminished quality of life and number of extra-articular manifestations and comorbidities especially cardiovascular diseases which result in increased mortality [1, 2]. The aetiology and pathogenesis of RA are not clearly defined. However, some genetic factors were found to be contributed to RA susceptibility. *HLA-DR* loci were the most associated gene with RA susceptibility, although *HLA-DR* accounts only for approximately one-third of the genetic predisposition to RA [3]. Other genes also contributed to the genetic susceptibility to RA are cytokine genes such as TNFα, IL-1β and IL-6 genes which account for the relatively small additional role in RA genetic predisposition [3].

Interleukin 6 (IL-6) is one of the most studied pro-inflammatory cytokines that have the important role in the pathogenesis of RA. Serum and synovial fluid levels of IL-6 and soluble IL-6 receptors (sIL6R) were found to be elevated in patients with RA [4]. IL-6 promotes the production of autoantibodies such as Rheumatoid Factor (RF) and Anti-Citrullinated Peptide Antibody (ACPA). In addition, it triggers the imbalance between Th17 cells and regulatory T cells (Treg). It also has a role in promoting synovial inflammation and cartilage and bone destruction as well as extra-articular manifestation including cardiovascular, psychological and skeletal disorders [5]. Blockade of IL-6 activity with a soluble anti-IL-6 molecule tocilizumab has been found to decrease the disease

activity as well as the radiological progression of RA [6].

The human *IL-6* gene is located on chromosome 7p21. Among the polymorphic sites described in the *IL-6* gene promoter, there are two biallelic polymorphisms that may be associated with differences in cytokine production: *−174G/C* (rs1800795) and *−572G/C* (rs1800796). These polymorphisms consist of a single nucleotide change from guanine (G) to cytosine (C) at positions −174 and −572 in the promoter region, respectively [7]. *IL-6* promoter polymorphisms have been associated with susceptibility to RA, however, conflicting results were observed in different populations [8, 9].

In the present study, we aimed to investigate genotype and allele frequencies of − 174 and −572 IL-6 promoter gene polymorphisms in Egyptian patients with rheumatoid arthritis in comparison to control group.

Subjects and Methods

Study design

This study was conducted on 99 Egyptian rheumatoid arthritis patients recruited from Internal Medicine and Rheumatology Clinic and Department of National Research Centre. The control group included 99 healthy Egyptian subjects with no family histories of any autoimmune diseases. The RA patients were diagnosed according to American College of Rheumatology (ACR) criteria [10]. Full medical history was taken and thorough clinical examinations were performed for all patients. Laboratory investigations including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) were performed. Also, Disease Activity Score (DAS) and Larsen score were calculated [11, 12]. Written informed consents were taken from patients and controls, and the study was approved by the National Research Centre ethics committee.

Methods

Blood collection and molecular analyses

Venous blood samples were collected in EDTA tubes and stored at −80 °C, till DNA extraction. Genomic DNA extraction from white blood cells was carried out by QIA gene extraction Kit.

Determination of IL-6 gene polymorphisms

The final volume of PCR reaction mixture was 25µl containing 40 ng genomic DNA, 10 picomoles each of forward and reverse primers at the concentration of 1X, 1X PCR master mixture.

To identify (−174) G/C polymorphism, the forward and the reverse primers were (5′-TTGTCAAGACATGCCAAGTGCT-3′) and (5′-GCCTCAGAGACATCTCCAGTCC-3′) and for (−572) G/C polymorphism, the forward and the reverse primers (5′-GGAGACGCCTTGAAGTAACTGC-3′) and (5′-GAGTTTCCTCTGACTCCATCGCAG-3′) were used. PCR amplification was carried out at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at (57°C and 55°C for 30 sec for −174G/C & −572G/C respectively), extension at 72°C for 30 sec, and final extension at 72°C for 7 min. The PCR product of −174G/C was digested by Fast digestion of 5 units of *Nla III* enzyme at 37°C yielded G allele (244 + 133 + 11) bp and C allele (133 + 111 + 56) bp products. For −572 G/C polymorphism, *MbiI* fast digestion restriction enzyme at 37°C was used yielded G allele (102, 61) bp and C allele (163) bp products. The obtained fragments' sizes were analysed on a 2% agarose gel [13].

Statistical Analysis

Data was analysed using the Statistical Program for Social Science (SPSS) for Windows, Version 16.0 Chicago, SPSS Inc., 2007. The statistical data are reported as the mean ± SE, frequencies and percentages when appropriate. Comparison between two means and more than two means was done using student t-test and ANOVA test respectively. Chi-square and Fisher's exact test were used to examine the relationship between different variables. A statistical significance was considered when $P \leq 0.05$ and Logistic regression was used for calculating the odds ratio.

Results

Comparison between RA patients and controls in selected characteristics are shown in Table 1. The female percent in RA patient was significantly higher than that in controls. The mean serum level of Anti- CCP of RA patients was significantly elevated to reach 25-fold that of controls. Also, the mean serum level of CRP of RA patients was significantly increased by 70% compared with controls. In addition, the mean serum levels of RF-IgA, IgG, and IgM were significantly elevated to reach (17-, 28-, and 34- fold, respectively) those of controls.

Table 2 show genotype and allele frequencies of *−572G/C* gene polymorphism in RA patients and controls. No statistical differences in genotype or allele frequencies between RA patients and controls were found.

Table 1: Comparison between RA patients and controls in selected characteristics

Variables	RA patients N = 99	Controls N = 99	P value
Female (%)	90 (90.9%)	58 (58.59%)	< 0.001
Anti- CCP, Mean ± SE	160.4 ± 16.47	6.52 ± 0.56	<0.001
CRP (mg/L), Mean ± SE	24.39 ± 3.31	14.17 ± 3.15	<0.05
RF IgA (IU/L), Mean ± SE	157.2 ± 29.15	9.37 ± 1.16	<0.001
RF-IgG (IU/L), Mean ± SE	354.8 ± 66.78	12.56 ± 1.45	<0.001
RF IgM (IU/L), Mean ± SE	175.6 ± 35.66	5.15 ± 0.93	<0.001

Table 3 show genotype and allele frequencies of *−174G/C* gene polymorphism in RA patients and controls. Regarding GC genotype, RA patients group showed higher frequency compared to control group (46.5% and 23.2% respectively) (P < 0.001, OR = 3.409).

Table 2: The genotype and allele frequencies of *−572G/C* polymorphism in RA patients group and controls

Genotype	RA patient N = 99 n (%)	Control N = 99 n (%)	P value	Odd ratio (95% CI)
GG	26 (26.3)	36 (36.4)		1.000 (Reference)
GC	64 (64.6)	58 (58.6)	0.177	1.528 (0.824-2.832)
CC	9 (9.1)	5 (5.1)	0.130	2.492 (0.748-8.308)
Allele				
G	116 (58.6)	130 (65.7)	0.147	1.351 (0.899-2.031)
C	82 (41.4)	68 (34.3)		

Also, there was higher frequency of CC genotype in RA group (9.1%) compared to control group (1%) (P = 0.001, OR = 15.341). In accordance to allele frequencies, C allele was found in higher frequency in RA patients group (32.3%) compared with controls (12.6%) (P < 0.001, OR = 3.305).

Table 3: The genotype and allele frequencies of *−174G/C* polymorphism in RA patients group and controls

Genotype	RA patient N = 99 n (%)	Control N = 99 n (%)	P value	Odd ratio (95% CI)
GG	44 (44.4)	75 (75.8)		1.000 (Reference)
GC	46 (46.5)	23 (23.2)	0.000	3.409(1.827-6.361)
CC	9 (9.1)	1 (1)	0.001	15.341(1.88-125.181)
Allele				
G	134 (67.7)	173 (87.4)	0.000	3.305(1.976-5.528)
C	64 (32.3)	25 (12.6)		

Discussion

Rheumatoid arthritis is a chronic inflammatory disease resulting from a cascade of immunological reactions. IL-6 is an acute phase cytokine contributes to host defence against infectious agents and tissue injuries by inducing immunological and hematopoietic responses. However, uncontrolled persistent production of IL-6 may lead to the development of several immune-mediated diseases. Thus, dysregulated persistent production of IL-6 has a crucial role in the pathogenesis of RA [14].

Regarding *IL-6 −572G/C* gene polymorphism, our study identified that the *GC* genotype is the most frequently genotype observed in both RA patients and controls and no significant differences were observed in the genotypes and alleles frequencies between RA patients group and controls (Table 2). Our results were in agreement with Zavaleta-Muñiz et al., 2013 who found no significant differences in allele or genotype frequencies of this polymorphism between RA and controls. But in contrast to our results, they found that the *GG* genotype was the most frequently observed genotype in the Mexican mestizo population among patients with RA and healthy controls (54% and 60.8% respectively) [7]. Also, Li et al., 2014a observed no association for *IL-6 −572G/C* gene polymorphism and RA in Chines Han population. And in agreement with our results, they observed that GC genotype was the most frequent genotype found in both RA patients and controls (48.8% and 45.9% respectively) [9]. Our findings also were in agreement with other studies in which they found no relationship between *IL-6 −572G/C* gene polymorphism and RA in different populations such as Taiwan and Turkish population [8, 15]. However, in contrast to our results Lo et al., 2008 found that the most frequent genotype in RA patients was *CC* genotype (60.8%) which was similar to the genotype distribution of the controls [15].

Regarding *IL-6 −174G/C* gene polymorphism, our results found that both *GC* and *CC* genotypes were associated with RA risk, and *C* allele increased the susceptibility of RA in the studied Egyptian population. Our results came in agreement with Li et al., 2014a who observed a statistically significant association for *IL-6 −174G/C* gene polymorphism and RA. They identified higher frequency of *C* allele in RA patients compared to controls (2.1% versus 0.5%) OR = 4.823 and P = 0.016 [9].

Moreover, in agreement with our findings, Li et al., 2014b identified that the frequency of *CC* genotype and *C* allele were significantly higher in RA patients compared to controls (2% versus 0.25% and 10.24% versus 0.88% respectively) (P < 0.001). Their results also showed a significantly increased risk of RA for the *CC* genotype and the *C* allele of *IL-6 −174G/C* gene polymorphism in Chines population [16].

On the other hand, our results were against different studies in which they found no differences in allele or genotype frequencies of *IL-6 −174G/C* gene polymorphism between RA and controls in a different population such as Mexican and Turkish populations [7, 8]. The controversy of results may be due to different origin or ethnicity.

IL-6 is a pro-inflammatory cytokine that has different pleiotropic activities including induction of acute phase proteins and stimulation of T and B cells, synoviocytes and osteoclasts. These inflammatory reactions result in cartilage and bone damage as well as other systemic manifestations [17]. Previous study

observed that polymorphisms in the promoter region of the *IL-6* gene may be responsible for changes in the transcriptional activity and the expression of IL-6 in serum and synovial tissue, which could, in turn, lead to greater inflammation and thus affect the clinical status of RA patients [18]. In other study, patients with the *−174G* allele showed higher rates of progression of erosive damage, although it was not statistically significant even in the presence of longer disease duration at baseline [19].

In conclusion, this study revealed a significant association of *IL-6 −174G/C* gene polymorphism and RA in Egyptian population with significantly higher frequencies of *GC* and *CC* genotypes and *C* allele in RA patients compared to controls. No association between *IL-6 −572G/C* gene polymorphism and RA was found in our cohort RA patients. Also, no correlations between different genotypes and all measured biomarkers were observed.

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