

Association of IL-6 genotypes with their serum levels among Iraqi patients with rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a complex autoimmune system disease and significant impact on the health of the population in our world. Numerous studies confirmed that genetic factors play a crucial role in the pathogenesis of RA. Interleukin-6 (IL-6) is a common pro-inflammatory cytokine that has several roles in a variety of pathophysiologic systems, most notably in the development of rheumatoid arthritis (RA). This study aims to investigate the association of polymorphism of the "IL-6 174 G/C rs(1800795) gene" to their serum levels among Iraqi patients. A total of 60 participants (40 confirmed rheumatoid arthritis patients and 20 controls) were selected using a convenient sampling method. "Genetic polymorphism of IL-6 174 G/C rs(1800795)" was carried out using RFLP-PCR. Serum levels of IL-6 were performed using by enzyme-linked immunosorbent assay technique (ELISA) using a Human- IL-6 kit. Based on the polymorphism of *IL-6 174 G/C rs(1800795)*, serum levels of IL-6 were higher among rheumatoid arthritis patients with CC and GC genotypes than that with GG genotypes (CC, 137.5336 ± 35 , GC 135.0622 ± 70.88476 and GG 65.5587 ± 1.77873 , $P < 0.05$). Furthermore, patients with GG genotype had the lowest IL-6 level with an insignificant difference compared to controls $1.65.5587 \pm 1.77873$ vs. 59.8203 ± 1.30673 , $P > 0.05$.

Keywords: (IL-6), RA , ELISA, , 174 G/C(rs1800795), PCR.

1.Introduction

Rheumatoid arthritis (RA) is a complex autoimmune system disease and significant impact on the health of the population in our world [1]. Numerous studies confirmed that genetic factors play a crucial role in the pathogenesis of RA [1]. Genome-wide association studies have rheumatoid

arthritis was one of the conditions for which several susceptibility genes and single nucleotide polymorphisms (SNPs) locations were identified and developed (RA). Increasing quantities of studies have suggested that genetic factors may play a key role in the development of RA (GWAS) [2,3]. Interleukin-6 (IL-6) is a common pro-inflammatory cytokine that plays several functions in a range of pathophysiologic systems, most notably in the development of rheumatoid arthritis. IL-6 is a cytokine that is produced by the body's own immune system (RA) [4]. According to the findings of the research, "polymorphic sites in the promoter region of IL-6, including 174 G > C (rs1800795) and 572G > C (rs1800796)", are linked to an increased risk of rheumatoid arthritis (RA) [5]. Nevertheless, contradictory results were discovered in other populations. This study aims to investigate the association of polymorphism of the "IL-6 174 G/C rs(1800795) gene" to their serum levels among Iraqi patients.

2. Materials and Methods

This study included (40) patients with RA (20 males, 20 females) whose ages ranged from 25 to 85 years, and (20) apparently healthy individuals(controls) subjects (10 males and 10 females) whose ages ranged from 25 to 85years. They were chosen from different hospitals in Wasit.

Five milliliters of blood were collected from all participants and placed in a tube without anticoagulant and placed in a centrifuge at a speed of 2000 rpm for 10 minutes. After that, the serum was withdrawn into an eppendorff tube 2ml and preserved after being labeled with deep freezing until further processed.

Using Restriction Fragment Length Polymorphism (RFLP) Polymerase Chain Reaction (PCR). the optimal conditions of interleukin 6 gene detection by PCR initial denaturation 94°C, 5 min. 1 Cycle. Denaturation 94°C ,40 sec,35 cycle. Annealing 60°C, 40sec.. 35Cycle..Extension-1,63°C, 40 sec, 35 cycle. Extension -2 ,72°C,10 min1 cycle.(IL-6)concentrations in sera were measured by "enzyme-linked immunosorbent assay (ELISA)" technique using Human- Interleukin-6 (Bioassay Technology Laboratory).

3. Results

3.1 Serum levels of IL-6 according to "IL-6 174 G/C" genotypes

Serum levels of IL-6 according to IL-6 174 G/C genotypes are shown in Table (3-1). Rheumatoid arthritis patients with CC and GC genotypes showed higher levels of IL-6 than that with GG genotype (CC, 137.5336±35.60066, GC 135.0622±70.88476 and GG 65.5587±1.77873, $P \leq 0.05$. There is a clear increase in the levels of this interleukin among patients with rheumatoid arthritis

who have the genotype CC, and these results agree with the previously mentioned results that demonstrate the association of the genotype with the predisposition to disease, as this genotype increases the possibility of developing rheumatoid arthritis. On the other hand, the patients with the GG genotype showed the lowest level of this interleukin among rheumatoid arthritis patients, and this supports the previous results, as this genotype does reduce the degree of predisposition to the disease.

Table 3.1 Serum levels of IL-6 according to “*IL-6 174 G/C*” genotypes

Parameters Groups	Pg/ml		
	GG	Mean ±SD GC	CC
Control	59.8203±1.30673	78.1270± 0.0	0.0
RA patients	65.5587±1.77873	135.0622±70.884 76	137.5336±35. *
P-value	0.986	0.695	-
Statistical significance	NS	NS	NS

NS :Non-significant P> 0.05

SD: Standard deviation

*p < 0.05

4. Discussion

Based on the polymorphism of *IL-6 174 G/C* gene , serum levels of IL-6 , the results reveal that although there were no significant differences, serum levels of IL-6 were higher among rheumatoid arthritis patients with CC and GC genotypes than that with GG genotypes (CC, 137.5336±35, GC 135.0622±70.88476 and GG 65.5587±1.77873, P < 0.05. Furthermore, patients with GG genotype had the lowest IL-6 level with an insignificant difference compared to controls. The results of this study were in agreement with Gaber et al., 2013[6] who found that serum IL-6 levels were significantly higher in RA patients compared to control (p = 0.04), particularly those with CC promoter polymorphism. In agreement, higher levels of IL-6 were present in serum,

synovial tissue and synovial fluid from patients with RA compared to those with non-inflammatory arthritis[7,8]. Chronic joint inflammation caused by RA results in the synthesis of IL-6 and its receptor, known as IL6R. IL6R is expressed on effector cells, which are the cells that are responsible for causing and prolonging inflammation. Interleukin-6 is overproduced in the synovial tissue of RA patients, leading to elevated quantities of the protein in both the serum and the synovial fluid [9]. On the other hand, allele 174 C is related to a decreased IL-6 promoter strength of expression, and subjects with the CC genotype have lower amounts of IL-6 in their serum compared to subjects with the GC or GG genotype [10]. Others stated that no definite association between gene polymorphisms and IL-6 serum levels was noticed [11]. RA patients with the IL-6 -174GG genotype, are known to have lower serum concentrations of IL-6 [12].

5. Conclusion

1. Polymorphism of the *Interleukin-6 (IL-6) 174 G/C rs(1800795) gene* can be associated with the serum levels of IL-6 among rheumatoid arthritis patients.

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