The Effects of Gene Polymorphisms in Interleukin-4 on the Susceptibility of Rheumatoid Arthritis in a Iraq Population

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Abstract

Rheumatoid Arthritis (RA) has been the most disease that recorded high number in Iraqi population, Interleukin-4 (IL-4) have been reported to associate with pathogenesis of rheumatoid arthritis (RA); which it is role of IL-4 genetic polymorphisms in RA. Method: A total of 50 unrelated IRAQ patients with RA and 40 healthy Iraqi volunteers with no family histories of any autoimmune diseases were recruited. The promoter IL-4-590 C/T polymorphisms were genotyped gene polymorphism implemented used RFLP technique. Result: The results show the genotype distributions and allele frequencies of IL-4-590 C/T polymorphisms in RA patients were significantly different from healthy. Statistically significant differences were observed in genotypes for IL-4-590. The frequencies of the T allele on the IL-4-590 were significantly increased in RA patients. Conclusion. The IL-4-590 promoter polymorphisms may be associated with increased risk of RA and could be used as genetic marker for assessing the susceptibility and severity of RA in Iraqi.

Keywords: IL-4 gene; RFLP; Rheumatoid Arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a disease of autoimmune disease that causes hope Worm in the joints and chronic inflammatory related with progressive disability. In autoimmune disease, a device begins Immune to your healthy tissues. In the case of rheumatoid arthritis, complications in some systems, early death, previous studies show that it was 0.5 to 2% of the world’s population [1].

RA pathogenesis refer to secretion many of the pro-inflammatory cytokines such as IL4, IL6 and ather [2, 3]. Furthermore, the HLA-DR loci were estimated to account for only about one-third of the genetic predisposition to RA [4]. A single-nucleotide polymorphism of ccr4 was found to be associated with susceptibility to rheumatoid arthritis in Japanese and Taiwanese population [5, 6]. Many cytokine genes were also playing an important role in its pathogenesis [7]. Interleukin-4 (IL-4) and interleukin-6 (IL-6) are the two most important cytokine genes associated with RA [8, 9].

IL-4 is the first discovered B-cell pleiotropic cytokine that promotes proliferation of T cells and antibodies production of B cells and plays an important role in the immune system [10, 11]. Therefore, polymorphisms affecting genes of IL-4 and IL-6 can be linked with RA risk and become of great interest to researchers [12]. IL-4-590 promoter polymorphism, a C-to-T base substitution, has been suggested to be associated with RA, especially with early pauciarticular juvenile rheumatoid arthritis [13]. Many previous studies examined the association of IL-4 gene polymorphisms with RA [14], but their data are conflicting, so the association of IL-4 gene polymorphisms with RA in Iraq could not be deduced and needs further studies.

2. Materials and Methods

2.1. Sampling and Data Collection

This a case-control study consisted of 50 patients with RA and 40 healthy persons without a history of immunological diseases as a control group. All subjects signed an informed consent, and clinical data of patients were collected from patient files and questionnaires. Our study was approved by the Research Ethics of the Iraqi Ministry of Health. About 2 ml of whole blood was collected from all subjects.

2.2. DNA Extraction and Purification

Genomic DNA was extracted from whole blood collected in EDTA-tubes from all subjects (patients and control individuals) using Genomic DNA Extraction Blood DNA Mini Kit (FAVORGENE). The concentration (ng/ml) and purity (260/280 nm) of the DNA extracts were measured at 260 nm and 280 nm with a NanoDrop spectrophotometer (OPTIZEN POP – Korea).
2.3. Genotyping

The candidate SNP (rs11209032) in the \textit{il4} gene at position IL4 -590C/T was investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The amplification reaction was done with 5 μl of 200ng/μl of genomic DNA, 12.5 μl of 1X Master Mix (Promega), 2 μl of 10 pmol of each specific primer pair (forward and reverse primer), and completed the volume of 25 μl PCR mixture with DNase free water.

The source of the primer was Bioneer (South Korea). Primer sequences of IL4 -590C/T were forward (5’-ACTAGGCCTCCTGATACG-3’) and reverse (5’-GTTGTAATGCTCAGTCCTCTG3’) [12]. The technique for PCR included a pre-denaturing temperature at 95°C for 1min, followed by 35 cycles at 94°C for 45 s; 63°C for 30 s; and 72°C for 1 min with a final extension at 72°C for 10 min. Ten μl of PCR products (487 bp in length) was digested according to Promega company protocol, which the digestion reaction mixture (36 μl) composed of 0.5 μl PsmF1, 2 μl of buffer B, 0.2 μl BSA buffer, 7 μlof 1X Muti core buffer and 16.3 μl of DH₂O; the reaction was incubated at 37°C for 16h. Subsequently, The product was separated on a 1.5% agarose gel for 45min and power 70 volt and 20mA. Finally, The gel was viewed by RedSafe™ Nucleic Acid Staining (iNtRON) under ultraviolet light. PsmF1 digestion of the PCR product yielded 252 bp for the undigested allele C/C, 60bp and 192bp C/T genotype was completely digested into 252, 192 bp, 60 bp and T/T genotype 252 bp fragments; all 4 fragments (252 bp, 192 bp, 60bp ) corresponded to the heterozygous C>T genotype (Figure 1). The frequency of allele calculated according to Hardy-Weinberg law and the statistical analysis implemented using odd ration at P-value <0.05.

3. Results and Discussion

The results of present study show that the demographic distribution was shown in table (1), the age means were 47.5±12.48for control and 38±10.37 for patients according to sex female more than male in patients with significant increment, male percentage was 35%, 18% in control and patients respectively and female percentage was 65%, 82% in control and patients respectively, this results deal with international studies that improved that female was more susceptible to the disease than male, this may be because the difference of hormonal activity between male and female also the deference’s of behavior and life style (12,13).

The results of study show IL4 genotype show significant variation between alleles in patient and control, the CC was appeared in 30%control while it was 40% in patients, TT genotype was more frequent in patients (34%) than control (12.5%). CT genotype was more frequent in control (47.5%) than patients (26%), (table, 2) (figure 1). The relative risk analysis show that there is significant variation between patient and control in CC and CT genotype at p-value 0.05 (table 3).

![Image](546x17 to 591x32)

Table-1. Some Characteristics of Study Groups

| rs3761548 Genotypes | Cases (No.=50) | Controls (No.=40) | Test | P-value |
|---------------------|---------------|------------------|------|---------|
| Age (year)          | 47.5±12.48    | 38±10.37         | t = 0.03* | 0.0 5   |
| Sex                 |               |                  |      |         |
| Male                | 18% (9)       | 14% (35)         | X² =0.06 | 0.0 5   |
| Female              | 82% (41)      | 26% (65)         |      |         |

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Table-2. Distribution of Allele Frequency and Genotype of IL4 in Case-Control Study

| Genotype | Patients N (%)(100%) | Control N%      | TEST X² | Odd ratio | CI 95%         |
|----------|----------------------|-----------------|---------|-----------|----------------|
| CC       | 20(40)               | 16(30)          | 0.02*   | 1.26      | 0.46-3.43      |
| CT       | 17(34)               | 5 (12.5)        | 1.53    | 0.55-4.18 |
| TT       | 13(26)               | 19(47.5)        |         |           |                |

OR: Odd Ratio.
CI: Confidence Interval.

Table-3. The Relative Risk of Genotyping in Study Groups

| Genotype | Relative risk | CI 95% | P-value |
|----------|--------------|--------|---------|
| CC       |              |        |         |
| CT       | 72           | 0.72 – 2.41 | 0.022* |
| TT       | 28           | 0.60- 1.97 | 0.44   |
Rheumatoid arthritis (RA) is a common chronic autoimmune disorder characterized by the destruction of articular cartilage and bone, which affects many of patients worldwide. In this study, we investigated whether IL-4 promoter polymorphisms influence the susceptibility of RA in an Iraqi population. Our results showed that the TT genotype carriers had markedly higher risk for RA compared with CC genotype carriers for IL-4 promoter polymorphisms, and the CC genotype carriers had markedly higher risk for RA; besides, the T allele of IL-4 promoter polymorphisms promoter polymorphisms had shown an association with susceptibility of RA in a Iraqi population.

IL-4 is one of anti-inflammatory cytokine, produced by activated CD4+ lymphocytes, mast cells, and basophils and exerts an important role in the immune system on different cell types [15]. In humans the IL-4 gene has been mapped to chromosome 14q32 [16].

The IL-4 gene promoter contains many of polymorphic loci, which were reported to influence the susceptibility of many diseases such as RA, including the IL-4-33C/T [17], IL-4-589C/T [18], and IL-4-590C/T/IL-4-590C/T were well studied and reported to be associated although the role of the genotype and allele frequencies of with many diseases, such as rheumatoid arthritis [19]. IL-4-gene in association with rheumatoid arthritis has been documented, we did not find any reports with regard to the genetic polymorphisms of IL-4-590C/T with rheumatoid arthritis patients in Iraq population. In this study, we firstly reported the role of genetic polymorphisms of IL-4 promoter in RA in Iraqi population. We found that IL-4-590C/T polymorphisms are associated with the RA risk, and the T allele of IL-4 promoter polymorphisms has significantly increased the susceptibility of RA in Chinese population. This finding suggests that the IL-4-590C/T polymorphisms may be used as a genetic marker for the onset and development of RA in Iraqi population.

This finding suggests that, besides the IL-4-590C/T polymorphisms may also be used as another genetic marker for the onset and development of RA in Iraqi population.

This study found that the genotype and allele frequencies of IL-4-590C/T polymorphisms are associated with the susceptibility of RA in a Iraqi population.

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