



## Single Nucleotide Polymorphism of *IL1B* Gene (rs16944) in a Sample of Rheumatoid Arthritis Iraqi Patients

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### Abstract

Cytokines play a prominent role in etiology and pathogenesis of rheumatoid arthritis (RA), and one of these cytokines is interleukin-1 $\beta$  (IL-1 $\beta$ ). The association between *IL1B* gene single nucleotide polymorphism (SNP: rs16944) and rheumatoid arthritis (RA) in a sample of Iraqi patients was investigated. Fifty-one RA patients (21 males and 30 females) were enrolled and their age range was 20 - 63 years ( $44.9 \pm 1.5$  years). In addition to patients, 45 apparently healthy control subjects were also enrolled in the study. They matched patients for ethnicity (Iraqis), gender (14 males and 31 females) and age ( $41.3 \pm 1.3$  years). Analysis of Hardy-Weinberg equilibrium (HWE) in RA patients and controls revealed that the *IL1B* genotypes were consistent with the equilibrium, and no significant differences ( $p > 0.05$ ) were observed between the observed and expected genotype frequencies. Inspecting *IL1B* genotype and allele frequencies in RA patients and controls revealed that there were no significant variations between these frequencies, although a decreased frequency of *T* allele (67.7 vs. 73.3%) and an increased frequency of *C* allele (32.3 vs. 26.7%) were observed in patients compared to controls. In conclusion, the results are in favor of no association between *IL1B* gene SNP (rs16944) and RA in Iraqi population.

**Keywords:** Rheumatoid arthritis, Interleukin-1 $\beta$ , Single nucleotide polymorphism.

## تعدد اشكال النيكلوتيدة المفردة لجين بين ابيضاض-بيتا ١ (rs16944) في عينة من مرضى التهاب المفاصل الرثوي العراقيين

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### الخلاصة

درست المصاحبة ما بين تعدد اشكال النيكلوتيدة المفردة لجين بين ابيضاض-بيتا ١ (rs16944) ومرض التهاب المفاصل الرثوي في عينة من المرضى العراقيين. شملت الدراسة ٥١ من مرضى التهاب المفاصل الرثوي (٢١ ذكور و ٣٠ اناث) وضمن المدى العمري ٢٠ - ٦٣ سنة ( $44.9 \pm 1.5$  سنة). وفضلا عن المرضى، فقد شملت الدراسة ايضا ٤٥ من افراد السيطرة الاصحاء ظاهريا والذين وافقوا المرضى من ناحية العرقية (عراقيين) والجنس (١٤ ذكور و ٣١ اناث) والعمر ( $41.3 \pm 1.3$  سنة). اظهر تحليل توازن هاردي-واينبرغ في مرضى التهاب المفاصل الرثوي و في افراد السيطرة الاصحاء ظاهريا والذين وافقوا المرضى من ناحية العرقية والجنس وضمن المدى العمري ٢٠ - ٦٣ سنة ( $41.3 \pm 1.3$  سنة) ان التواتر الجيني لمختلف الجينات كان متوافقا مع التوازن الجيني، ولم يلاحظ فروق ذات دلالة احصائية ( $p > 0.05$ ) بين التواتر الجيني المرصود والتواتر الجيني المتوقع. فحص جينوتيب و تواتر اليلات جين *IL1B* في مرضى التهاب المفاصل الرثوي و في افراد السيطرة الاصحاء ظاهريا والذين وافقوا المرضى من ناحية العرقية والجنس وضمن المدى العمري ٢٠ - ٦٣ سنة ( $41.3 \pm 1.3$  سنة) اظهر ان مرضى التهاب المفاصل الرثوي لديهم تواتر اليل *T* اقل (٦٧.٧٪) وتواتر اليل *C* اقل (٣٢.٣٪) مقارنة بمرضى السيطرة الاصحاء ظاهريا والذين وافقوا المرضى من ناحية العرقية والجنس وضمن المدى العمري ٢٠ - ٦٣ سنة ( $41.3 \pm 1.3$  سنة).

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واينبيرغ في مرضى التهاب المفاصل الرثوي وافراد السيطرة بان الطرز الوراثية لتعدد اشكال النيكلوتيدة المفردة لجين بين ابيضاض-بيتا ١ كانت متناغمة مع التوازن ولم تكن هناك فروق ذات دلالة احصائية (الاحتمالية < ٠.٠٥) بين التكرارات المشاهدة والمتوقعة للطرز الوراثية. وعند تقصي تكرار الاليلات والطرز الوراثية لتعدد اشكال النيكلوتيدة المفردة لجين بين ابيضاض-بيتا ١ في مرضى التهاب المفاصل الرثوي وافراد السيطرة، لم تكن هناك فروق ذات دلالة احصائية، بالرغم من نقصان تكرار الاليل T (٦٧.٧ مقابل ٧٣.٣%) وزيادة تكرار الاليل C (٣٢.٣ مقابل ٢٦.٧%) في المرضى مقارنة بالسيطرة. استنتجت الدراسة بعدم وجود مصاحبة ما بين تعدد اشكال النيكلوتيدة المفردة لجين بين ابيضاض-بيتا ١ (rs16944) ومرض التهاب المفاصل الرثوي لدى العراقيين.

## Introduction

Rheumatoid arthritis (RA) is a worldwide disease with a global prevalence of 0.24% as based on the Global Burden of Disease in 2010, while among Eastern Mediterranean populations; it has been estimated to be 0.37% [1]. It is a chronic inflammatory autoimmune disease characterized by joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality [2]. Etiologically, the cause of RA is unknown, but substantial evidence suggests that the disease develops in individuals after interaction between inherited genetic risk factors and environmental triggers. Such interaction can lead to immune dysregulations that are identified as autoantibodies and disturbance of cytokines in serum many years prior to the diagnosis of disease [3]. Accordingly, cytokines have been suggested to play a prominent role in the etiology and pathogenesis of RA, and one of these cytokines is interleukin-1 $\beta$  (IL-1 $\beta$ ) [4].

IL-1 $\beta$  belongs to the IL-1 family, which consists of three further interleukins (IL-1 $\alpha$ , IL-18, and IL-33). Expression of IL-1 $\alpha$  occurs on the surface of the same cell or retains within the cell, while biological actions of IL-1 $\beta$  is produced by acting on other cells [5]. The action of both interleukins can be blocked by an endogenous inhibitor, which is IL-1 receptor antagonist (IL-1Ra). Both types of IL-1 (IL-1 $\alpha$  and IL-1 $\beta$ ) exert their activity by binding and signaling through two types of cellular receptors; IL-1RI and IL-1RII. The binding of IL-1 to the former receptor results in intracellular signal transduction, while the latter receptor functions as a decoy receptor for IL-1 [6].

In RA patients, IL-1 $\beta$  showed a significant increased level in serum and synovial fluid; and moreover, such increase was positively correlated with the disease severity [7]. A further effect of such cytokine was reported on the capacity of synovial fibroblast to produce cytokines, chemokines, and prostaglandins. In addition, IL-1 $\beta$  can activate osteoclast, and in RA patients, such activation was associated with increased expression of endothelial cell adhesion molecules, and the resulting effects lead to an imbalance in bone metabolism that favors bone resorption and osteoporosis [8]. These observations suggest a role for IL-1 in the pathogenesis of RA.

Genetic studies that were based on single nucleotide polymorphisms (SNPs) of *IL1* genes revealed that genetic polymorphisms of *IL1A*, *IL1B*, and *IL1RN* genes may have a role in susceptibility to RA. Among these is *IL1A* gene polymorphism at positions -899 (C/T) and +4845 (G/T), which showed positive associations with RA (i.e. increased the risk to develop the disease), and moreover, they were associated with altered serum levels of IL-1. Two *IL1B* gene SNPs (-511 C/T and +3953 C/T) were also associated with an increased risk of RA and impacted the disease activity and IL-1 $\beta$  expression [9]. In Indian patients, the *IL1B*<sub>-511</sub> C allele was reported to have a protective effect against RA development, and in addition, the results suggested a possible role of *IL1B*<sub>+3953</sub> CT genotype in the severity of RA [10]. However, these findings were subjected to the effect of the ethnicity of RA patients. Such subject has been inspected by a study that was based on a meta-analysis of 16 published association studies of *IL1A*, *IL1B* and *IL1RN* gene polymorphisms in RA. For *IL1B*<sub>-511</sub> SNP (C/T), a negative association between T allele and RA was reported in Caucasian patients, while in Asian RA patients, no such association was observed. However, the TT+TC genotype of *IL1B*<sub>-3953</sub> SNP (C/T) showed a positive association between Caucasian and Asian RA patients [11]. In Tunisian RA patients, *IL1B*<sub>-511</sub> SNP (C/T) was suggested to be associated with susceptibility and severity of disease [12].

The present study aimed to evaluate the association between a SNP in *IL1B* gene (T/C: rs16944) and RA among Iraqi patients.

## Materials and Methods

### Patients

The study was approved by the Ethics Committee at the Iraqi Ministry of Health, in which 51 Iraqi RA patients (21 males and 30 females) were enrolled and their age range was 20 - 63 years ( $44.9 \pm 1.5$  years). They were referred to the Rheumatology Clinic (Baghdad Teaching Hospital) during November 2015 - June 2016 for diagnosis and treatment. The diagnosis was made by the consultant medical staff at the Rheumatology Unit, and it was based on the revised diagnostic criteria established by the American College of Rheumatology (ACR), 2010 [13]. In addition to patients, 45 apparently healthy controls were also enrolled in the study. They matched patients for ethnicity (Iraqis), gender (14 males and 31 females) and age ( $41.3 \pm 1.3$  years).

### Methods

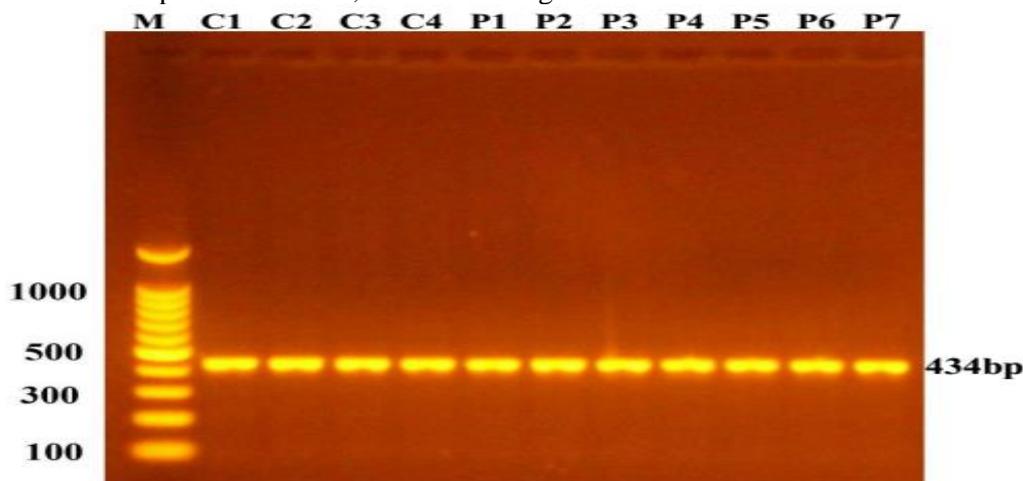
The Genomic DNA was extracted from EDTA blood using ReliaPrep™ Blood gDNA Miniprep System (Promega Corporation, USA), and after assessing purity and concentration, it was subjected to PCR amplification. Two primers were designed (Forward: 5'-TTCCAGCCTTCTTTGTTTTGTCTC-3' and Reverse: 5'-TCCTCCTCTCTGAGCTTCTACTC-3') for genotyping of *IL1B* gene SNP (T/C: rs16944) by using Geneious software version 10.1.3. The PCR reaction was performed in a final volume of 25  $\mu$ l; which included 12.5  $\mu$ l GoTaq green Master mix, 0.75  $\mu$ l forward primer (10  $\mu$ M), 0.75  $\mu$ l reverse primer (10  $\mu$ M), 2  $\mu$ l DNA sample (50 ng) and 9  $\mu$ l nuclease-free distilled water. The PCR conditions were initial denaturation at 95°C for 5 minutes (1 cycle), followed by 35 cycles of denaturation at 95°C (30 seconds), annealing at 60°C (30 seconds) and extension at 72°C (30 seconds), followed by a final extension at 72°C for 7 minutes. The amplified PCR fragments were sent for Sanger sequencing using ABI3730XL automated DNA sequencer (Macrogen Corporation – Korea). The genotypes were revealed by Geneious software after alignment with a reference sequence in the Gene Bank.

### Statistical analysis

Allele and genotype frequencies were given as percentage frequencies. The genotype frequencies were first tested for their agreement with Hardy-Weinberg equilibrium (HWE), and a significant difference between the observed and expected genotype frequencies was assessed by Pearson's Chi-square test (<https://www.easycalculation.com/health/hardy-weinberg-equilibrium-calculator.php>). The association between *IL1B* gene SNP (T/C: rs16944) and RA was presented in terms of odds ratio (OR), etiological fraction (EF) and preventive fraction (EF), and a significant difference was assessed by two-tailed Fisher exact probability [14]. The software WinPepi version 11.65 was used to carry out the latter calculations.

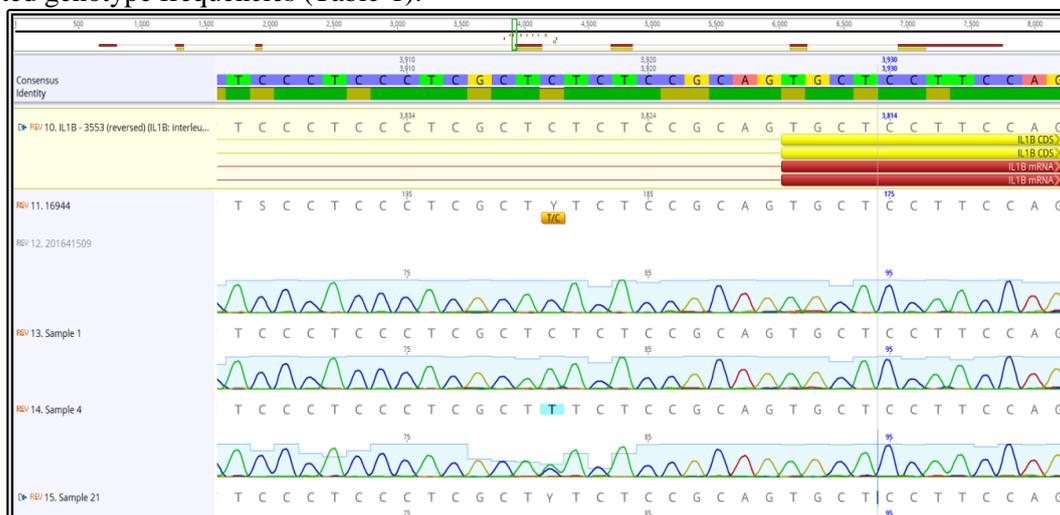
### Results

Agarose gel electrophoresis of the *IL1B* gene PCR amplified products (SNP rs16944) showed a single a band of 434bp molecular size, as shown in Figure-1.



**Figure 1**-Gel electrophoresis of *IL1B* gene PCR products (SNP rs16944) on 1% agarose at 5 v/cm<sup>2</sup> for 45 minutes showing bands of 434bp (Lane M: 100bp DNA ladder; Lanes C1-C4: samples of controls; Lanes P1-P7: samples of rheumatoid arthritis patients).

The SNP rs16944 (T/C; Chromosome 2: region 112,836,790-112,837,790bp) was presented with three genotypes (TT, TC and CC) and two alleles (*T* and *C*) (Figure-2). Analysis of Hardy-Weinberg equilibrium (HWE) in RA patients and controls revealed that the genotypes were consistent with the equilibrium, and no significant differences ( $p > 0.05$ ) were observed between the observed and expected genotype frequencies (Table-1).



**Figure 2-** DNA sequence chromatogram of *IL1B* gene SNP (T/C: rs16944) showing three genotypes: TT (sample 4), TC (sample 21; Y) and CC (sample 1). In addition, the reference sequence (rs1800629) is also given.

**Table 1-**Numbers and percentage frequencies (observed and expected) of *IL1B* gene (rs16944SNP) genotypes and their Hardy-Weinberg equilibrium (HWE) in rheumatoid arthritis patients and controls.

Genotype	Rheumatoid Arthritis Patients (No. = 51)				Controls (No. = 45)			
	Observed		Expected		Observed		Expected	
	No.	%	No.	%	No.	%	No.	%
TT	26	51.0	23.3	45.7	26	57.8	24.2	53.8
TC	17	33.3	22.3	43.7	14	31.1	17.6	39.1
CC	8	15.7	5.3	10.4	5	11.1	3.2	7.1
HWE Analysis	$X^2 = 2.900$ ; D.F. = 1; $p > 0.05$				$X^2 = 1.883$ ; D.F. = 1; $p > 0.05$			

Inspecting *IL1B* genotype and allele frequencies in RA patients and controls revealed that there were no significant variations between these frequencies, although a decreased frequency of *T* allele (67.7 vs. 73.3%) and an increased frequency of *C* allele (32.3 vs. 26.7%) were observed in patients compared to controls (Table-2)

**Table 2-**Statistical analysis of association between genotypes and alleles of *IL1B* gene (rs16944 SNP) and rheumatoid arthritis

Genotype or Allele	Patients (No. = 51)		Controls (No. = 45)		Odds Ratio	95% Confidence Interval.	<i>p</i>
	No.	%	No.	%			
TT	26	51.0	26	57.8	0.76	0.34 - 1.69	NS
TC	17	33.3	14	31.1	1.11	0.47 - 2.59	NS
CC	8	15.7	5	11.1	1.49	0.46 - 4.87	NS
<i>T</i>	69	67.7	66	73.3	0.76	0.41 to 1.42	NS
<i>C</i>	33	32.3	24	26.7	1.32	0.71 - 2.45	NS

*p*: Probability; NS: Not significant ( $p > 0.05$ )

## Discussion

Inspecting rs16944 SNP (T/C) of *IL1B* gene in RA patients and controls revealed no significant difference in the distribution of genotype and allele frequencies, although a decreased frequency of *T* allele and an increased frequency of *C* allele were observed in patients. Such SNP has been investigated by other studies in RA patients, but inconsistent observations have been made. Bax *et al.* [9] reported that *IL1B* gene SNP (-511 T/C) was associated with an increased risk of RA, and such SNP was also associated with disease activity and expression of IL-1 $\beta$ . In contrast, the *IL1B*<sub>-511</sub> *C* allele was reported to have a protective effect against RA development in Indian patients [10]. These different observation might be related to the ethnicity of RA patients. Such subject has been inspected by a study that was based on a meta-analysis of 16 published association studies of *IL1B* gene polymorphisms in RA. A negative association between *IL1B*<sub>-511</sub> *T* allele and RA was reported in Caucasian patients, while in Asian RA patients, no such association was observed [11]. In Tunisian RA patients, *IL1B*<sub>-511</sub> SNP (T/C) was suggested to be associated with susceptibility and severity of disease [12]. More recently, the SNP *IL1B*<sub>-511</sub> showed a significant association with chronic periodontitis and RA in Mexican patients [15]. In conclusion, the results are in favor of no association between *IL1B* gene SNP (rs16944) and RA in Iraqi population.

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