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The Allelic and Polymorphism Association of Tumor Necrosis Factor-alpha Gene (-308 G/A Genotype) in Some Iraqi Rheumatoid Arthritis Patients

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Abstract

This study aims to determine whether -308 G/A polymorphism in tumor necrosis factor-alpha gene (TNF-α), is associated with susceptibility to rheumatoid arthritis in Iraqi population of TNF-a. This case-controlled study was performed including 42 RA patients (15 males, 27 females; mean age 55,67years; range 30 to 72 years) and 30 healthy controls (10 males, 20 females; mean age 52,8 years; range 30 to 70 years). We determined the frequency of -308 G/A TNF-a gene polymorphism by amplification refractory mutation system-polymerase chain reaction restriction fragment length polymorphism (ARMS-PCR). Also, we determined the association of TNF-α -308 G/A polymorphism with rheumatoid arthritis in Iraqi patients. There wassignificant difference between genotype frequency of -308 G/A polymorphism and rheumatoid arthritis. In RA patients, the genotype frequency of -308 G/Apolymorphism was GG (11.90%), GA (45.24%), and AA (42.86%). In the control group, the genotype frequency of -308 G/A polymorphism was GG (3.33%),GA (83.33%), and AA (13.33%). Statistical analysis showed significant association in the genotype frequency of this AA polymorphism in RA patients ($p<0.009$*), and GG genotype bot no significant ($p=0.390$). There was significant difference in genotype frequency of this GA polymorphism in control ($p<0.001$*). Our findings demonstrate that the TNF-α-308 G/A gene polymorphism may represent a significant risk factor for rheumatoid arthritis in Iraqi population and there is association between the TNF-α-308 G/A polymorphism and rheumatoid arthritis patients.

Keywords: TNF-α; Polymorphism; rheumatoid arthritis.

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1. Introduction

Rheumatoid arthritis (RA) is a autoimmune disease characterized by chronic inflammation of the joints tissue [1]. which may lead to structural damage of the bone and cartilage, that is associated with extra-articular, articular and systemic effects; including kidney, spleen, lungs, eyes and heart [2]. It is estimated that the disease affects 0.5 to 2% of the world's population [3].This disorder is three times more frequent in females than males. The Prevalence of disease increases with age and is highest in females older than 65 years [4].

The tumor necrosis factor alpha (TNF-α) have roles in the pathobiology of rheumatoid arthritis. It is a major pro-inflammatory and anti-inflammatory cytokines; exert their influence, depending on the time, cellular location and extent of release. TNF-α is a axial cytokine in rheumatoid arthritis, lead to enhancement of apoptosis and regulation of cell proliferation [5]. TNF-a is produced mostly by activating macrophages although it can be produced by T CD4+ cells and natural killer cells [6].

The TNF gene is located on chromosome 6 (chromosome 6p21.3) within the major histocompatibility complex (MHC).The expression high of TNF-α responsible for ROS (reactive oxygen species) released in Rheumatoid arthritis, this lead to damage of patient tissues, in addition to inflammation [7].There are different genetic variations in TNF-α gene such as ARMS (Amplification refractory mutation system) and SNPs (single nucleotide polymorphisms), these variations affect on TNF-α gene expression. Polymorphism TNF-α gene in codon -308 G (guanine) has been reported in different autoimmune diseases as Rheumatoid arthritis. The genetic variation in codon -308 results in presence of two allelic features (G and A) in which G is defines as the common variant, and the presence of A(adenine) defines as less common.A allele displays increase TNF-α-308 gene transcription expression to produce 6-7 folds as compared to G allele [8].

The study aim is evaluating association of TNF-α -308 G/A polymorphism with rheumatoid arthritis in Iraqi patients.

2. Materials and Methods

Rheumatoid arthritis Patients and controlstotal of 72 (42 Patients and 30 control) subjects visiting Abu-Graeeb hospitals and Al-Shaheed Saif Saad healthy center, Baghdad, Iraq were recruited (during 2016–2017) for this study. The diagnosis of RA consisted of 42 unrelated Iraqi rheumatoid arthritis patients [male = 15, female = 27, age range 30 to 72 years (mean age 55,67 ± 1,65 years)]. The control group consisted of 30 matched, unrelated healthy blood donors [male = 10, female = 20, age range 30 to 70 years (mean age 52,8 ± 2,10 years)] from the same Iraqi population.

Genomic DNA extraction

Genomic DNA was extracted from 200 µl of whole blood using the ReliaPrep™Blood gDNAMiniprep System kit (promega). Concertation and purity ware detected using nanodrope.

Cytokine genotyping

The TNF-α geneA and G alleles at positions -308 were genotype using the amplification refractory mutation system (ARMS-PCR) reaction. The PCR Primers sequences were used in present study are shown in table (1).The PCR conditions for ARMS Technique were in table (2).(Depending on [9] and [10] sources). Electrophoresis was dose by using 2 % agarose gel concentration for 1 hour, 75 Vol, stained with ethidium bromide.

Table 1: The primers sequenceof TNFα (-308G/A) genotype

Gene Locus	**Sequence of Generic primer (antisense)**	**Sequence of Sense primers**
TNFα (G-308A)	5′-TCT CGG TTT CTT CTC CAT CG-3′	**G allele:** 5′-ATA GGT TTT GAG GGG CAT GG-3′ **A allele**: 5′-AAT AGG TTT TGA GGG GCA TGA-3′

Table 2: The ARMS-PCR Technique using for TNFα (-308G/A) genotype.

Subjects	**Cycles**	**Heat**	**S Time**
Pre-denaturation	1	94	5 min
Denaturation	10	94	15 sec
Annealing		65	50 sec
Extension		72	40 sec
Denaturation	25	94	20sec
Annealing		59	50 sec
Extension		72	50 sec
Final extension	1	72	7 min

3. Statistical analysis

The differences in allele/genotype (TNF-α -308G/A) frequencies and significant between patients and controls were analyzed bythe Fisher's exact test,Pvalues less than 0.05, were considered significant. The strength of the association of disease with respect to a particular allele/genotypeis expressed by odd ratio (OR), and Confidence

Intervals (CI) interpreted as *relative risk* (RR) following the Woolf's method as out lined by Schallreuter and his colleagues [11]. It was calculated only for those alleles/genotypes which were increased or decreased in rheumatoid arthritis patients as compared to control group. The RR was calculated for all the subjects using the formula given below:

RR = (a) × (d)/(b) × (c).

a = number of patients with expression of allele orgenotype.

b = number of patients without expression of alleleor genotype.

c = number of controls with expression of allele orgenotype.

d = number of controls without expression of allele or genotype.

Etiologic Fraction (EF):

The EF indicates the hypothetical genetic component ofthe disease. The values .0.0–0.99 is of significance. EF was calculated for positive association only where

RR.1 using the following formula [12].

EF = (RR-1)f/RR, where f = a/a+c

Preventive Fraction (PF):

The PF indicates the hypothetical protective effectof one specific allele/genotype for the disease. PFwas calculated for negative association only whereRR, 1 using the following formula [12].

PF = (1-RR)f/RR (1-f) + f, where f = a/a+c

Values,1.0 indicate the protective effect of the allele/genotype against the manifestation of disease**.**

4. Results and discussion

The results of present study show significant variation between alleles and genotype in patients and control.

The genotype frequencies of TNFα -308G/A polymorphism in rheumatoid arthritis patients and control individuals ware presented in Table 1. The homozygous AAgenotype was more frequent and significantly in rheumatoid arthritis patient's 42.86% and odds ratio (OR) 4.88,p=0.009*, Compared to control 13.33%. The frequency of GG genotype was less in patients and control (11.90% and 3.33% sequentially), while heterozygous GA genotype was found in 45.24% of the rheumatoid arthritis patient's against 82.33% of controls (OR= O.17. P= 0.001*). The homozygous GG genotype and heterozygous GA genotype for TNFα -308

frequency in Iraqi data were similar to those reported in Saudi Rheumatoid [13], Egyptian [14], Tunisian [15] and Turkish [16] populations. In addition to the genotype frequency all for GG, GA and AA data were similar to Iraq populations [17]. Table 2 shows the alleles frequencies of TNFα -308G/A polymorphism in rheumatoid arthritis patients and control individuals.The frequency of alleles A and G was none significantly in patients and control p= 0.228 (OR= 1.55 and 0.64 sequentially), in addition the frequency A allele was higher in rheumatoid arthritis patient's as compared to the control. The alleles-308G/A polymorphism has been related to increased transcriptional activity of the TNF gene [18]. The search for a function for the -308G/A polymorphism the expression of TNF has produced controversial results [19]. However, the association of the TNF-α allele with susceptibility to play a central role in the development of a more severe form of rheumatoid arthritis [20].The prominent role played by tumor necrosis factor alpha in inflammation and its relevance to both infectious and autoimmune diseases, has led to great interest in both the regulation of the TNF alpha gene, and the possibility that variants of the gene or deregulation of its production may be associated with rheumatoid arthritis [21]. have also recently reported an association of the -308G/A TNF polymorphism with the severity of the disease [22]. The firststudy of the _308 G/A Single nucleotide polymorphisms in relation to rheumatoid arthritis found no association between the frequency of the Single nucleotide polymorphisms and the incidence of rheumatoid arthritis [23]. Two subsequent Spanish studies alsofound no association between either _308G/A Single nucleotide polymorphisms and susceptibility to rheumatoid arthritis [24]. A large study of Japanese rheumatoid arthritis patients (n¼ for 545 patients) showed that while _308A allele was increased in rheumatoid arthritis patients [25]. had reported an increase in the _308A allele in RApatients, two recent studies from Taiwan30 and Sweden [26]. Reported an increased incidence of the _308G allele in rheumatoid arthritis patients, when compared to controls. In addition to studies of the incidence of rheumatoid arthritis, some researchers have examined the possible role of the allele and genotype variants in disease severity [27]. The study showed a association between the TNF-α gene in Loci -308 G/A polymorphism and rheumatoid arthritis. Show allele A and AA genotype associated links with rheumatoid arthritis. AA genotype frequency more than four times in TNF-α gene for Iraqi patients (table 3). This genotype is Etiologic Fraction in rheumatoid arthritis for Iraqi patients, While GG genotype Preventive Fraction in disease.

Table 3: Genotype frequencies of TNF-α -308 G/A polymorphism in rheumatoid arthritis patients and controls

Genotype	Rheumatoid arthritis(n=42) N(%)	Control(n=30) N(%)	P-value (Two-tailed)	OR	CI 95%
GG	11.90	3.33	0.390	3.92	0.45 to 34.20
GA	45.24	83.33	0.001*	0.17	0.05 to 0.51
AA	42.86	13.33	0.009*	4.88	1.47 to 16.16

Table 4: allelic frequencies of TNF-α -308 G/A polymorphism inrheumatoid arthritis patients and controls

Genotype	Rheumatoid arthritis(n=42) N(%)	Control(n=30) N(%)	P-value (Two-tailed)	OR	CI 95%
A	65.48	55.00	0.228	1.55	0.79 to 3.04
G	34.52	45.00	0.228	0.64	0.33 to 1.26

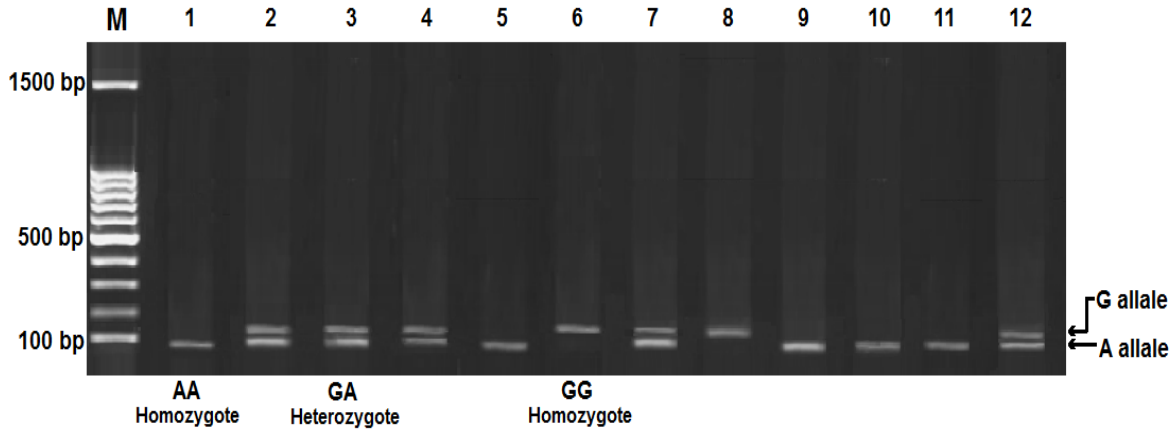

Figure 1: Electrophoresis for TNF-α -308 G/A genotype in same in rheumatoid arthritis patients, lane M DNA marker (100 to 1500 bp), lane 1,5,9,10,11 AA genotype, lane 2,3,4,7,12 GA genotype, lane 6,8 GG genotype.

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