

Association between genetic polymorphism in tumor necrosis factor-alpha gene and adverse effects of etanercept in rheumatoid arthritis patients

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ABSTRACT

Background. Gene polymorphisms affect etanercept's pharmacokinetics, pharmacodynamics, and side effects. This effect is evidenced by the extensive genetic variation in the drug's targets.

Objectives. This study aims to find the association between different genotypes of the promoter region of the TNF- α gene at -308G/A(rs1800629), -857C/T(rs1799724), -863 C/A(rs1800630), -1031 T/C (rs1799964), -806 C/T (rs4248158) and -376 G/A (rs1800750) and the side effects of ETN that occurred to Iraqi RA patients.

Method. The trial included patients with rheumatoid arthritis who had been using ETN for at least six months. The participants were from the Baghdad Teaching Hospital Rheumatology Unit. The PCR was sequenced to determine the polymorphism in the TNF promoter region at sites -308 G/A (rs1800629), -857 C/T (rs1799724), -863 C/A (rs1800630), -1031 T/C (rs1799964), and -376 G/A (rs4248158) (rs1800750). The link between the genetic variation at these loci and the etanercept's most frequent adverse effect was then investigated.

Results. The only genotype of (-376 G/A) significantly related to an increased risk of upper respiratory tract infection is the GG genotype, according to the results of this study. However, genotypes for the remaining SNPs did not demonstrate a statistically significant association between ETN and an increased risk of upper respiratory tract infections, injection site response, or skin rash in patients.

Conclusion. This study revealed that only the GG genotype of (-376 G/A) was significantly associated with an elevated risk of upper respiratory tract infection.

Keywords: adverse effects, etanercept, rheumatoid arthritis, genetic polymorphism, TNF- α

INTRODUCTION

Over the past two decades, rheumatoid arthritis (RA) patients have had access to various therapy options. This diversity is crucial since many patients will require many drugs to attain and maintain optimal disease management during the duration of their illness [1].

Tumor necrosis-alpha (TNF- α) antagonists are regarded as the most effective approach for treating a number of rheumatic diseases, including RA. The fully humanized dimeric fusion protein etanercept

(ETN) is among the most effective TNF antagonists. Etanercept has been demonstrated to promote remission, reduce disease activity, and prevent clinical and radiological disease progression in RA patients, resulting in significant improvements in symptoms, function, and quality of life [2].

Etanercept is a well-tolerated biological drug [3] that provides a long-term therapy option with sustained effectiveness and excellent safety in clinical practice [4]. Most often reported adverse effects are injection site reactions, rhinitis, and upper respiratory tract infections [3].

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Pneumonia, upper respiratory infection, abscess, bronchitis, gastroenteritis, septic arthritis, sepsis, peritonitis, and wound infections were among the serious illnesses related to ETN usage [5].

Regarding the influence of ETN on the cardiovascular system, the results are contested. Moreover, several studies suggested that long-term usage may be related to an increased risk of cardiovascular disease (CVD), such as acute myocardial infarction (MI) [6,7]. Contrastingly, some research revealed that there was no increase in CVD-related mortality with TNF- α antagonist therapy [5].

To improve the clinical and economic efficacy and decrease the adverse effects of RA treatment, scientists are attempting to build a genetic testing methodology for many biologic medications' personalization [8]. Gene polymorphisms influence the pharmacokinetics, pharmacodynamics, and adverse effects of etanercept, as proven by the numerous polymorphisms of this drug's targets [8].

Although the genetic polymorphism influence of the TNF- α gene on the clinical response to etanercept has been extensively explored [9-12], neither Iraqi nor international researchers have investigated the association between ETN adverse effects and genetic polymorphism in the TNF- α gene. Accordingly, this study aims to find the association between different genotypes of the promoter region of the TNF- α gene at -308G/A(rs1800629), -857C/T (rs1799724), -863 C/A (rs1800630), -1031 T/C (rs1799964), -806 C/T (rs4248158) and -376 G/A (rs1800750) and the side effects of ETN that occurred to Iraqi RA patients.

PATIENTS AND METHODS

Study design

This paper is a part of a larger study [10,13] which performed with a suitable convenient sample of eighty Iraqi RA patients with established RA according to the revised 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) Classification Criteria for RA [14].

All study participants were recruited from the Rheumatology Unit at the Baghdad Teaching Hospital in Baghdad, Iraq. This unit supports various Iraqi populations, including rural, urban, and inner-city locations from multiple governorates.

All patients were identified and treated under the supervision of specialists at the Rheumatology Unit of Baghdad Teaching Hospital (Baghdad, Iraq) between October 12, 2020, and August 8, 2021.

Patients selection

Ninety-seven individuals met the inclusion criteria with active RA utilizing ETN alone as a single therapy. However, only eighty-six patients agreed to participate in the trial, and only eighty patients fulfilled the study's

requirements. Written consent was obtained from all participants.

The inclusion criteria

- 1 - Patients must be diagnosed with rheumatoid arthritis according to revised 2010 ACR/EULAR RA classification criteria [14].
- 2 - Patients with high disease activity as measured by disease activity score based on 28 joints and ESR (DAS28-ESR), i.e., DAS28-ESR should be greater than 5.1 at baseline.
- 3 - Patients were also required to have taken ETN regularly for a minimum of six months with no history of missing doses.

Exclusionary criteria

- 1 - Patients who have used ETN for less than six months or longer than a year.
- 2 - Patients having other connective tissue disorders.
- 3 - Patients that combine ETN with extra DMARDs.
- 4 - Inadequate data.

Data collection

Data on demographic characteristics (age, weight, disease duration, recent lab data such as ESR, tender and swollen joints, patients' and evaluators' VAS scores, and ETN side effects during the previous six months of usage) were gathered through patient interviews using a patient information chart designed specifically for this study.

Collection and preparation of samples

Five milliliters of venous blood were extracted from the forearm vein of each participant. Two milliliters of blood were transferred to a tube containing ethylene diamine tetra acetic acid (EDTA) for DNA extraction.

DNA extraction

Using the Promega DNA extraction Kit, DNA from blood samples was purified. PCR was employed for enzymatic amplification with Master Taq polymerase enzyme and a hybrid thermal cycler following DNA extraction.

The Primer

The TNF gene DNA sequences were obtained from the NCBI GenBank database. The program Primer Premier 3 was used to construct PCR primers (Table 1) with a melting temperature of (62° C), a primer length of (21) nucleotides, and a PCR amplicon length of (966).

TABLE 1. The primer sequences, Annealing temperature, and Product size (bp)

Primer name	Sequence	Annealing Temp. (°C)	Product size (bp)
TNF- α _1-F	5'-TGTA AACGACGGCCAGTCTCAGAGAGCTTCA GGGATA-3'	60	96
TNF- α _1-R	5'-CAGGAAACAGCTATGACCGGGACACACAAGCA TCAA-3'		6

TNF- α _1-F: the forward primer. TNF- α _1-R: the reverse primer

TABLE 2. Participants' demographic information and clinical features

Category	value
Age(years)	58.74 \pm 21.88
Gender	Male n (%)
	Female n (%)
Weight(kg)	50.58 \pm 11.177
Disease duration (years)	9.34 \pm 5.539
Baseline ESR (mm/h)	58.74 \pm 21.885
ESR after 6 months (mm/h)	39.19 \pm 25.305
Baseline DAS28	5.82 \pm 0.44
DAS28 after 6 months	4.55 \pm 1.39

Results are reported as means \pm SD or count (percentage).

WBC: White blood cell counts; ESR: Erythrocyte sedimentation rate; DAS28: Disease activity score in 28 joints.

Sequencing of PCR products

PCR product was sequenced using the Sanger technique and a DNA analyzer (ABI3730XL) (Macrogen Corporation – Korea). The findings were collected by email and processed using Geneious Prime (V 2021.1.1) software (Biomatters Ltd., Auckland, New Zealand; www.geneious.com). The types of genotyping in the promoter region of the TNF- α gene at -308G/A(rs1800629), -857C/T(rs1799724), -863 C/A(rs1800630), -1031 T/C(rs1799964), -806 C/T (rs4248158) and -376 G/A(rs1800750) then identified.

Statistical Analysis

The SPSS for Windows 26.0 program was utilized for data analysis (SPSS, Inc., Chicago, IL, USA). Continuous variables were reported as the mean \pm standard deviation (SD) of the values. Allele and genotype percentages and frequencies were provided. A probability of 0.05 or less was considered statistically significant. Using the Shapiro–Wilk test, the normality of the results was determined. The Chi-square test or Fisher's exact test was employed to examine proportional group differences. Fisher's exact test was utilized if one of the predicted values in a 2 \times 2 comparison was less than 5.

RESULTS

Demographic, disease, and baseline clinical characteristics variables of all participants are presented in Table 2.

Prevalence of genotypes polymorphism in all patients.

Figure 1 and 2 illustrates the high prevalence of the GG genotype for both -308 G/A and -376 G/A, as well as the high prevalence of the G allele, in Iraqi RA patients enrolled in the current study. While the TT genotype was more predominant in over half of patients with -1031T/C, with a high frequency of the T allele (97.5 %) and a low proportion of the C allele (30 %), as illustrated in Figure 2.

Notably, the CC genotype was the most frequent for both the -857 C/T and -863C/A variants. Additionally, the C allele was the most abundant in both locations.

Almost (90%) of patients had the CC genotype for the -806 C/T variation, with the C allele being present in (100%) of cases and the T allele being present in just (10 %) of patients, as illustrated in Figure 2.

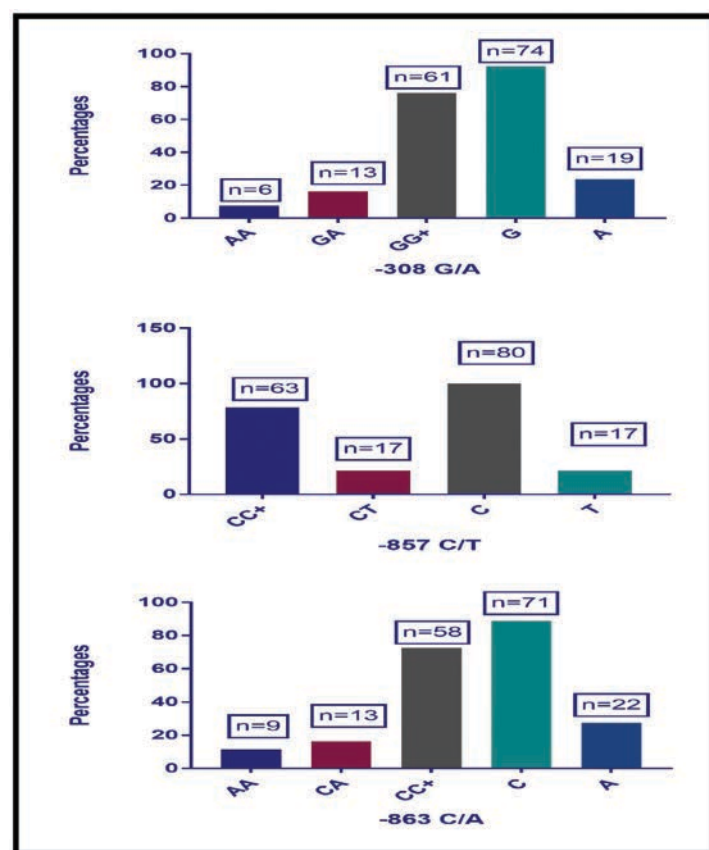


FIGURE 1. Proportion of genotypes and alleles for TNF- α promoter region -308G/A, -857C/T, and -863C/A polymorphisms. Total n=80.

+: The wild type

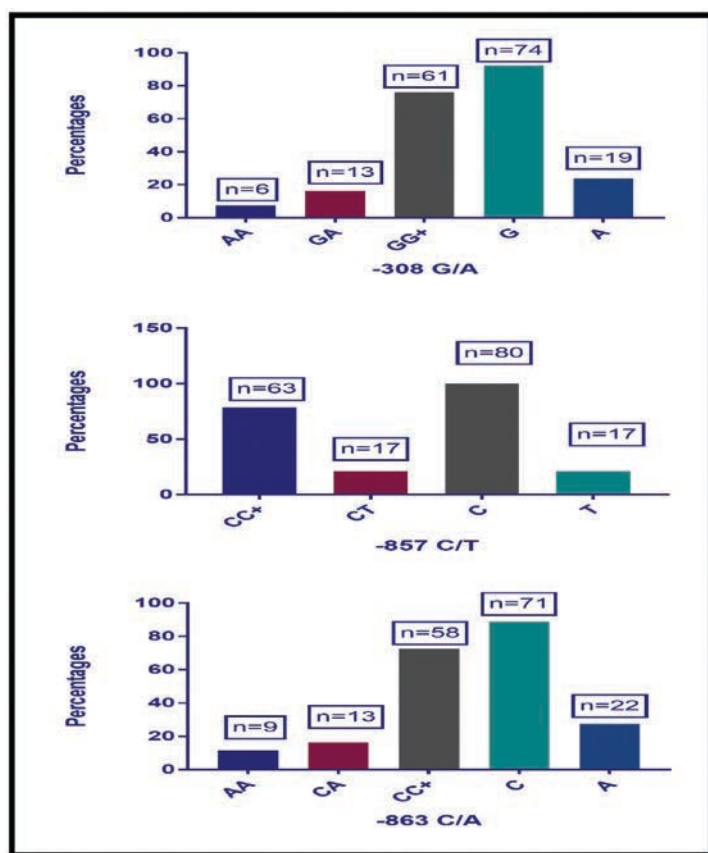


FIGURE 2. Proportion of genotypes and alleles for TNF-α promoter region -1031T/C, -806C/T, and -376G/A polymorphisms. Total n=80. +: The wild type

TABLE 3. The relationship between specific genotypes and the increase in the occurrence of upper respiratory tract infections associated with ETN

Genotypes					
-308G/A	Genetic variant	AA	GA	GG°	P. Value
	NO. (%) of patients with S/E	0(0)	2(2.5)	6(7.5)	0.8
-857 C/T	Genetic variant	CC°	CT		
	NO. (%) of patients with S/E	6(7.5)	2(2.5)		0.65
-863C/A	Genetic variant	AA	CA	CC°	
	NO. (%) of patients with S/E	0(0)	2(2.5)	6(7.5)	0.72
-1031T/C	Genetic variant	CC	TC	TT°	
	NO. (%) of patients with S/E	1(1.25)	2(2.5)	5(6.25)	0.39
-806 C/T	Genetic variant	CC°	CT		
	NO. (%) of patients with S/E	6(7.5)	2(2.5)		0.22
-376 G/A	Genetic variant	GA	GG°		
	NO. (%) of patients with S/E	2(2.5)	6 (7.5)		0.048*

N= 8 patients, Fisher exact was the test used to identify the statistical difference between the groups. °: The wild genotype

The relationship between specific genotypes and some adverse effects associated with etanercept use.

To determine whether SNPs in the promoter region of the TNF-α gene affect the type of side effects associated with ETN use, we examined the association between specific genotypes and the occurrence of upper respiratory tract infections, injection site reaction, and skin rash, which were the most frequently reported side effects by the study’s participants.

The association between genotype and increase in the occurrence of upper respiratory tract infections.

This study confirmed that only the GG genotype of (-376 G/A) is significantly correlated with an increased risk of developing upper respiratory tract infection. While the remaining genotypes of other SNPs did not show a significant difference, as shown in Table 3.

The association between genotypes and injection site reaction.

Table 4 highlights no significant difference between genotypes with an increased risk of developing injection site reactions.

The association between genotypes and the development of skin rash.

As shown in Table 5, there was no statistically significant difference between any genotypes associated with an increased risk of skin rash.

DISCUSSION

TNF-α is a highly polymorphic gene with several polymorphic sites. Several SNPs may influence the secretion of specific cytokines involved in the pathophysiology and the susceptibility to RA in several sites, especially in promoter regions, as proven in previous studies [15,16].

The results of this study in a sample of 80 RA patients treated with ETN revealed six polymorphic sites in the promoter region of the TNF-α gene (-308 G/A, -376 G/A, -806 C/T, -857 C/T, -863 C/A, and -1031 C/T).

Although, previous studies [10,13,17–19] conducted in Iraq have explored the association between a polymorphism in the promoter region of TNF-α and RA in a sample of Iraqi patients. However, no previous study has examined the association between these SNPs and adverse effects of ETN.

Globally, many studies have examined the effect of a combination of SNPs in the TNF-α gene on the response of several anti-TNF-α [20].

TABLE 4. The relationship between specific genotypes and injection site reaction associated with ETN

Genotypes					
-308G/A	Genetic variant	AA	GA	GG°	P. Value
	NO. (%) of patients with S/E	0 (0)	4(5)	14(17.5)	0.44
-857 C/T	Genetic variant	CC°	CT		
	NO. (%) of patients with S/E	15(18.75)	3(3.75)		0.68
-863C/A	Genetic variant	AA	CA	CC°	
	NO. (%) of patients with S/E	1(1.25)	4(5)	13(16.25)	0.58
-1031T/C	Genetic variant	CC	TC	TT°	
	NO. (%) of patients with S/E	1(1.25)	6 (7.5)	11(13.75)	0.71
-806 C/T	Genetic variant	CC°	CT		
	NO. (%) of patients with S/E	15(18.75)	3(3.75)		0.41
-376 G/A	Genetic variant	GA	GG°		
	NO. (%) of patients with S/E	2(2.5)	16(20)		0.17

N= 18 patients, Fisher exact test was used to identify the statistical difference between the groups. °: The wild genotype

TABLE 5. The relationship between specific genotypes and the development of skin rash

Genotypes					
-308G/A	Genetic variant	AA	GA	GG°	P. Value
	NO. (%) of patients with S/E	0 (0)	2(2.5)	4(5)	0.54
-857 C/T	Genetic variant	CC°	CT		
	NO. (%) of patients with S/E	4 (5)	2(2.5)		0.39
-863C/A	Genetic variant	AA	CA	CC°	
	NO. (%) of patients with S/E	1(1.25)	2(2.5)	3(3.75)	0.56
-1031T/C	Genetic variant	CC	TC	TT°	
	NO. (%) of patients with S/E	1(1.25)	1(1.25)	4(5)	0.20
-806 C/T	Genetic variant	CC°	CT		
	NO. (%) of patients with S/E	5(6.25)	1(1.25)		0.662
-376 G/A	Genetic variant	GA	GG°		
	NO. (%) of patients with S/E	1(1.25)	5(6.25)		0.173

N= 6 patients, Fisher exact test was used to identify the statistical difference between the groups. °: The wild genotype

For instance, The SNPs -857C/T, -308G/A, -238G/A, and +489G/A in the TNF- α gene and their association with therapeutic efficacy were evaluated by Chatzikyriakidou et al. [21] in 58 RA patients taking infliximab.

Similarly, an association study of three TNF- α related SNPs (-308G > A, -238G > A, and -857C > T) was

conducted in 280 RA patients of Caucasian origin treated with TNF-inhibitors like etanercept, adalimumab, and infliximab [22].

Furthermore, some meta-analyses have been conducted to investigate the connection between numerous SNPs and TNF-inhibitor responsiveness, including the -308 A/G polymorphism, the -857 C/T polymorphism, and the -238 A/G polymorphism [23,24].

The anti-TNF- α field is characterized by costly medications, wide response variability, hazardous consequences, and reasonably substantial knowledge about the molecular targets of these therapies [25].

Even though ETN is generally well-tolerated and safe, it can produce adverse drug reactions [26]. Pharmacogenetics may enable us to minimize the overall risk of adverse events and promote safety [26].

In other words, an improved understanding of the reasons behind these side or adverse effects may allow for better medication tailoring and a reduction in needless toxicity in patients receiving TNF- α inhibitors [26].

It is generally established that the most often reported adverse events associated with ETN are injection site reactions, skin rash, rhinorrhea, and upper respiratory infections [3].

According to the current study's findings, the most prevalent adverse events were upper respiratory tract infections, injection site reactions, and skin rash development. Examining the association between these adverse effects and genetic polymorphisms in the TNF- α promoter region revealed one significant correlation between -376 G/A and the possibility of acquiring upper respiratory tract infections. Patients with the -376 GG genotype were more susceptible to upper respiratory tract infection than those with the GA genotype.

Nevertheless, no causal association has been established in the current study. Additional long-term researches with a higher number of patients are required to elucidate the significance of this relationship.

Moreover, to comprehend this finding, we must first understand that linking these infections entirely to anti-TNF- α medications is difficult because individuals with chronic inflammatory disorders such as RA have a roughly two-fold increased risk of infection than the general population regardless of therapy type [27]. In addition, most patients eligible for anti-TNF- α therapy are already on corticosteroids and/or other immunosuppressive medications, which have raised their risk of infection [27].

However, given TNF's critical function in immune defense against invading pathogens, it is biologically

likely that inhibiting TNF- α might increase infection incidence [28].

There was no previous research to compare the results because this was the first study examining the link between the genetic variation in the promoter area of TNF- α and the most common side effects of ETN.

CONCLUSION

Examining the relationship between the adverse effects of ETN and genetic polymorphisms in the TNF- α promoter region revealed a significant link between -376 G/A and the risk of developing upper respiratory tract infections. Upper respiratory tract in-

fection was more prevalent in patients with the -376 GG genotype than those with the GA genotype.

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Authors' contributions: The first author conducted the research as part of his Ph.D. thesis, which was supervised by the second and third authors. All authors contributed to the manuscript's creation and revision for the final draft.

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