RESEARCH ARTICLE

Association between polymorphisms within the gene coding for tumor necrosis factor (TNF)-alpha with outcomes of treatment in a sample of Iraqi patients with ankylosing spondylitis taking etanercept: an observational study [version 1; peer review: awaiting peer review]

Shaimaa Saleh Khudhur[1], Eman Saadi Saleh[2], Mohammed Hadi Alosami[3], Laith G. Shareef[4]

[1]Department of Clinical Pharmacy, University of Baghdad, College of Pharmacy, Baghdad, 10011, Iraq
[2]Department of Clinical Laboratory Sciences, University of Baghdad, College of Pharmacy, Baghdad, 10011, Iraq
[3]Department of Rheumatology, University of Baghdad, College of Medicine, Baghdad, 10011, Iraq
[4]Department of Pharmacy, Al-Rasheed University College, Baghdad, 10011, Iraq

v1 **First published:** 23 Dec 2022, **11**:1576
https://doi.org/10.12688/f1000research.128842.1
Latest published: 23 Dec 2022, **11**:1576
https://doi.org/10.12688/f1000research.128842.1

Abstract

Background: Ankylosing spondylitis (AS) is a progressive, chronic inflammatory illness with an unclear etiology that explicitly targets the vertebral column, peripheral joints, and extraarticular tissues. The purpose of this research was to investigate if the existence of single nucleotide polymorphisms (SNPs) in the promoter region of the tumor necrosis factor-alpha (TNF-α) gene at positions -1031T/C (rs199964), -857C/T (rs1799724) and -806C/T (rs4248158) in a sample of Iraqi AS patients could influence the patients' outcomes with etanercept.
Methods: Sixty patients with established AS receiving only etanercept were selected to enroll in this study, with a mean age of 40.75±8.67 years; 51 patients were male. Patients were classed as "responders" if they obtained a *Bath Ankylosing Spondylitis Disease Activity Index* (BASDAI) 50 clinical response and as "non-responders" if they did not achieve a BASDAI 50 clinical improvement after at least six months of treatment. After polymerase chain reaction (PCR) product amplification of the purified blood DNA, the promoter region of TNF-α gene SNPs was established by Sanger sequencing.
Results: This research found a significant difference in the TT genotype of rs1799964, P = 0.02, in the responder group, in contrast to the TC genotype of rs1799964, which was significantly more frequent in the non-responder group, P = 0.01. The wild TT genotype of rs1799964 seemed to enhance the probability of being a responder.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

Any reports and responses or comments on the article can be found at the end of the article.

Nevertheless, the heterozygote TC genotype of rs1799964 showed a negative and significant correlation for responsiveness to etanercept. **Conclusion**: The TT genotype of rs1799964 is associated with a higher likelihood of responding to ETN, suggesting that it is a valuable diagnostic for predicting response in Iraqi AS patients.

Keywords
ankylosing spondylitis, genetic polymorphism, TNF-α, SNPs, etanercept

Corresponding author: Laith G. Shareef (Laithalkunani@yahoo.com)

Author roles: Saleh Khudhur S: Conceptualization, Data Curation, Formal Analysis, Methodology, Project Administration, Writing – Original Draft Preparation; **Saadi Saleh E**: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – Review & Editing; **Hadi Alosami M**: Conceptualization, Methodology, Software, Supervision, Writing – Review & Editing; **G. Shareef L**: Software, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Saleh Khudhur S, Saadi Saleh E, Hadi Alosami M and G. Shareef L. **Association between polymorphisms within the gene coding for tumor necrosis factor (TNF)-alpha with outcomes of treatment in a sample of Iraqi patients with ankylosing spondylitis taking etanercept: an observational study [version 1; peer review: awaiting peer review]** F1000Research 2022, **11**:1576 https://doi.org/10.12688/f1000research.128842.1

First published: 23 Dec 2022, **11**:1576 https://doi.org/10.12688/f1000research.128842.1

Introduction

Ankylosing spondylitis (AS) is a progressive, chronic inflammatory illness with an unclear etiology[1]. Prolonged inflammation can cause vertebral fusion, which may result in immobility[2]. Clinically, the sickness manifests as pain and increasing rigidity of the spine[3]. The hallmarks of AS are inflammation of the sacroiliac joints (SIJ) and spine[4]. AS is directly proportional to the prevalence of human leukocyte antigen (HLA)-B27 in a given population, and has been estimated to range between 0.1 and 1.4% in various parts of the world[5]. In Iraq, the estimated frequency of AS is 0.9 per 100,000, while HLA-B27 positivity was elicited in 55% of the population investigated[6]. In Arab people, between 2% and 5% of individuals are HLA-B27 positive, which is prevalent in 64% of AS patients[7]. TNF-α is a highly potent pro-inflammatory molecule and a critical component of the immune system's signaling cascade that is strongly activated in response to infection or tissue injury. Patients with AS, in particular, respond well to TNF antagonist therapy[8]. Etanercept (ETN) has been examined in various rheumatologic disorders[9]. ETN is the only soluble TNF receptor the Food and Drug Administration (FDA) has licensed for therapeutic use. It is typically administered in weekly doses of 50 mg subcutaneously (or 25 mg twice weekly), either by self-injection or by a caregiver[10].

Since the promoter region of the TNF-α gene would seem to be remarkably polymorphic, genetic variation is one of the most significant factors in the response[11]. In contrast to numerous aspects that could potentially influence or change the ETN response, genetic manifestations do not change during a patient's lifetime[12]. Consequently, polymorphism testing assists in dividing individuals with just a high response from those with an inappropriate or poor response. Accordingly, early identification of individuals who would not adhere to biological treatments allows a quicker transfer to another type of medicine, increasing the patient's likelihood of achieving therapeutic purposes more quickly[13]. The goal of pharmacogenomic and pharmacogenetic research is to use available knowledge about gene variants that influence the medication response to develop personalized treatment methods that enhance therapeutic effectiveness and safety[14]. Numerous single nucleotide polymorphisms (SNPs), primarily in the TNF promoter, are susceptible to rheumatoid arthritis (RA) and spondyloarthritis, and related to tumor necrosis factor inhibitor (TNFi) response. Genetic variations in cytokine genes may affect cytokine gene transcription and secretion, modulating cardiovascular disease development risk[15]. In addition, these promoter SNPs are thought to contribute to treatment responses by modifying TNF gene expression[16]. Identifying pharmacogenetic markers, enabling treatment only for those patients who will respond without the risk of unwanted effects, would considerably improve treatment efficacy and reduce expenses[17]. Previous studies have not examined the impact of the rs4248158C/T polymorphism on AS patients' response to ETN. No prior research has been conducted in Iraq to explore the influence of SNPs within the TNF-α promoter region upon the propensity to respond to ETN in AS patients. This current research study aimed to determine whether the presence of SNPs in the promoter region of the TNF-α gene at positions -1031T/C (rs199964), -857C/T (rs1799724), and -806C/T (rs4248158) in a sample of Iraqi AS patients could influence the patients' outcomes with ETN.

Methods

Ethical considerations

The research ethics committee approval (approval number: RECACPUB-3102020D) was obtained from the Scientific and Ethical Committee at the College of Pharmacy, Baghdad University, Iraq. This research was conducted in line with the Helsinki Declaration[18]. Before data collection, written consent was obtained from each of the participants.

Study design

An observational study was carried out using a suitable convenient sample of 60 Iraqi patients who were determined to have AS in accordance with the modified New York Criteria[19].

Setting

The participants in this study were recruited from the Rheumatology Unit of Baghdad Teaching Hospital at Medical City in Baghdad, Iraq. From January to December 2021, all patients were evaluated and treated by a rheumatology specialist, and 76 patients with established AS were receiving only an ETN prefilled pen containing 50mg manufactured by Pfizer Company given by subcutaneous injection once a week. A total of 95 participants were selected to enroll in the study. However, only 60 subjects met the study's participation criteria, with a mean age of 40.75±8.67 years; 51 patients of them were male, and only nine patients were female.

Variables

A 50% improvement in disease activity score (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) 50) was defined as a response to therapy[20]; patients were classed as "responders" if they obtained a BASDAI 50 clinical response and as "non-responders" if they did not achieve a BASDAI 50 clinical improvement.

Sample size

The number of subjects was determined using the G*Power program (RRID: SCR 013726) edition 3.1.9.7. The size of the sample has to be a minimum of 59 individuals with a 95% confidence interval, 90% power, a two-tailed alpha of 0.05, and an effect size of 0.40 (f).

Eligibility criteria

Patients age 18 and above who had been diagnosed with AS using the modified New York criteria[19] and had been taking ETN for at least six months, without a history of missed doses.

Exclusion criteria

Patients with coexisting other connective tissue conditions, recurring infectious diseases, cancer, hepatic or renal dysfunction, endocrine system inadequacies, hematological and cardiovascular diseases, or multiple sclerosis, any patient who has

been taking ETN for less than six months, and any patient taking disease-modifying antirheumatic drugs (DMARDs) in addition to ETN. In addition, any patient with insufficient data was eliminated.

Bias
Sampling flaws may arise during the research sample selection process. This is most noticeable in retrospectively longitudinal studies, when individuals' exposures and results have already happened before they are included. Nevertheless, sampling error is much less likely since the result is unknown at the time of enrollment. The ideal study population is well-defined, easily accessible, trustworthy, and capable of producing the needed results. To avoid bias, participants were recruited so that no age group or gender was preferred over others. We considered demographics and other information about participants (and nonparticipants) to help us adjust overall conclusions.

Data collection
Demographic data (such as age, weight, height, disease duration, smoking status, family history, presence of extraarticular manifestations (such as psoriasis, inflammatory bowel disease, uveitis, and osteoporosis) and ETN side effects) were obtained through direct interviews with patients utilizing a patient records sheet specially designed for this research project. The body mass index (BMI) was computed by dividing the weight in kilograms by the square height in meters[21].

Blood sample collection and preparation of specimens
For DNA extraction, 2 ml of the obtained blood specimen was transformed into an EDTA tube.

Clinical evaluation
A direct interview was performed with all patients in this study to evaluate disease manifestations, symptoms, medical history, and laboratory findings. In addition, the patient's disease activity was assessed by calculating BASDAI and Bath Ankylosing Spondylitis Functional Index (BASFI).

DNA extraction
The Promega ReliaPrep™ System for Genomic DNA (Promega Corp., USA) provides a practical approach for extracting DNA from blood samples. Enzymatic amplification was accomplished using a hybrid thermal cycler and conventional polymerase chain reaction (PCR).

The primers
The DNA sequences of the TNF-α gene were obtained from the GenBank database of NCBI. Primer Premier 3 software (RRID:SCR_003139) was utilized to generate PCR primers, as shown in Table 1, with a primer length of (21to20) nucleotides and a melting temperature of (60°C). As a result, the PCR amplicon length of the TNF-α gene was (949) base pairs (bp).

PCR amplification
The PCR process involves three major steps: denaturation, annealing, and extension.

The first step depicts DNA denaturation at high temperatures (90–97°C). Step two is to anneal the primers to the DNA template strands in order to prime extension. Step three involves extending the annealed primers to make a complementary copy strand of DNA; the last extension step is performed as a verification stage[22]. As seen in Figure 1, the PCR results were segregated on 1.5% agarose gel electrophoresis stained with ethidium bromide and observed using a Gel imaging system. Macrogen Corporation, Korea, delivered the PCR products to ABI3730XL, an automated DNA sequencer, for Sanger sequencing. The data were sent to the researcher, who subsequently examined them using Geneious prime software (RRID: SCR_010519).

The summary of chemicals and kits used in this study are summarized in Table 2.

Statistical analysis
IBM SPSS Statistics (RRID: SCR_016479) version 27 software for Microsoft Windows was used throughout the statistical analysis process. The variance or dispersion of a collection of data values was quantified by expressing continuous variables as the mean standard deviation. Presenting discrete variables by their number and percentage, the direct count revealed the percentages and frequencies of alleles and genotypes. P-values less than 0.05 were considered significant, those less than 0.01 were considered highly significant, and those less than 0.001 were considered highly significant. The Shapiro–Wilk test was used to assess the findings′ normality. To determine the

Table 1. The sequences, annealing temperature, and product size (bp) of the primers.Note: F means the forward primer, and R is the reverse primer.

	Reference locus sequences (5′ - 3′)	Temperature annealing step(°C)	Size of products (bp)
Tumor necrosis factor-α-F	5`-TGTAAAACGACGGCCAGTGCTGACCAAGAGAGAAAGAAG-3`	55	949
Tumor necrosis factor-α-R	5`-CAGGAAACAGCTATGACGAGTCCTTGAGGGAGAGAAA-3`		

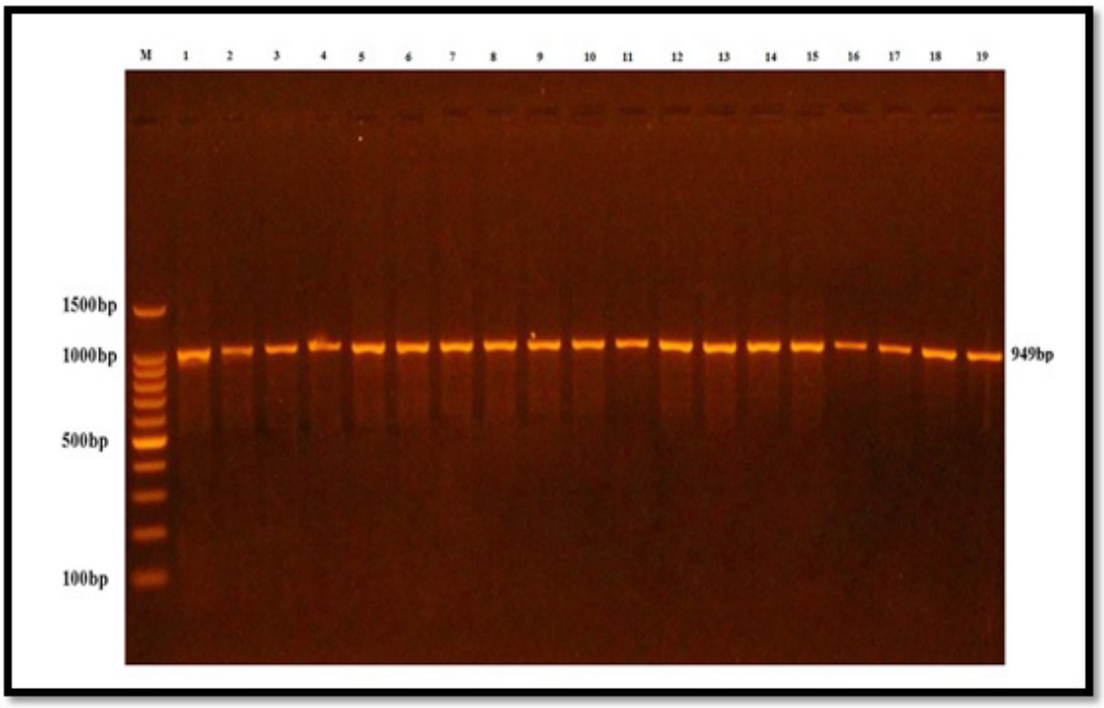

Figure 1. The amplification of the TNF-α gene of human samples was fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromide.M:100bp ladder marker. Lanes1-19 resemble 949bp PCR products.

Table 2. The sequences, annealing temperature, and product size (bp) of the primers.Note: F means the forward primer, and R is the reverse primer.

Chemical	Provider	Cat.No.	REF No.
TNF-α ELISA kit	Cusabio, China	CSB-E04740h	LOT:H25223454
hs-CRP ELISA kit	Demeditec, Germany	LOT:1110221	DE740011
Go Tag Green Master Mix	Promega, USA	M7822	NA
Agarose	Promega, USA	LOT:0000381091	V3121
Ethidium Bromide Solution (10mg/ml)	Promega, USA	LOT:0000337437	H5041
TAE 40X	Promega, USA	LOT:0000428	V4281
ReliaPrep™ Blood gDNA Miniprep System	Promega, USA	A5081	NA
100bpDNA Ladder	Promega, USA	PR-G2101	G210A
Column Wash Solution	Promega, USA	LOT:0000402367	A503B

significance of the difference between the means of two independent samples, an unpaired T-test was used. One-way analysis of variance (ANOVA) was used to determine the degree of difference between the groups under study .The chi-square test and the Fisher's exact test were used to organize and analyze the data. The phi correlation coefficient (phi) was used to establish the association between each genotype and the likelihood of becoming a responder. The influence of the genotypes on the chance of becoming a responder was estimated using a binary logistic regression analysis.

Results

Demographic data and clinical characteristics variables of the research participants are expressed in Table 3 and Table 4.

As shown in Table 5, there was a high prevalence of CC genotype for both rs1799724 C/T and rs4248158C/T in contrast to a low proportion of mutant C allele (2%) in rs1799964T/C. At the same time, the wild T allele in rs1799964T/C was more frequent and predominant in over half of patients (73%).

Furthermore, highlighting the disparity in genotypes frequency between responder and non-responder groups, the analysis of this study showed a significant difference in the TT genotype of rs1799964 (p-value = 0.02) in the responder group, in contrast to the TC genotype of rs1799964, which was significantly more frequent in the non-responder group (p-value = 0.01), as revealed in Table 6.

Table 7 shows the significant and non-significant association with each studied SNP in the TNF-α promoter regain gene by binary logistic regression. The TC-rs1799964 genotype significantly reduced the tendency to be a responder to ETN (OR=0.208, 95%CI:0.057-0.762, p=0.018); this indicates that the heterozygote TC of rs1799964 is a predictor for non-responsiveness to ETN in AS patients.

Similarly, the phi correlation coefficient (phi) analysis was shown to verify the strength of association between each genotype and the predisposition to be a responder to ETN. For example, Table 8 shows that the homozygote wild TT genotype of rs1799964 seemed to enhance the probability of being a responder to ETN in AS patients. Nevertheless, the heterozygote TC genotype of rs1799964 showed a negative and significant correlation for responsiveness to ETN.

Adverse events and associated SNPs of ETN therapy

To investigate whether SNPs in the TNF-α promoter region alter the severity of harmful effects from ETN use, we looked at the relationship between genotype and the occurrence of these adverse events: infection, injection site reaction, headache, and rash. These three SNPs were compared for each genotype between non-adverse and negative cases. Table 9 shows there was a non-significant difference between genotypes for an increased risk of developing an infection, injection site reaction, headache, and adverse rash effects.

Discussion

AS is a chronic inflammatory condition with an unknown cause. In contrast to other systemic autoimmune diseases, the innate immune system plays a dominant role in AS, which is characterized by the aberrant activity of innate and innate-like immune cells, such as T cells, group 3 innate lymphoid cells,

Table 3. Demographic data and the disease characteristicsof the participants.

Variables		R (n=33)	NR (n=27)	p-value
Age (years)		40±7.9	41.5±9.7	0.559[a]
Gender	**Male n(%)**	32(97%)	19(70.4%)	0.008*[b]
	Female n(%)	1(3%)	8(29.6%)	
BMI (Kg/m²)		28.4±5.6	29.7±5.3	0.340[a]
Smoking status(n%)	**Nonsmoker**	10(30.3%)	16(59.3%)	0.077[c]
	Active smoker	18(54.5%)	9(33.3%)	
	Ex-smoker	5(15.2%)	2(7.4%)	
Disease duration category	**< 5 year n(%)**	17(51.5%)	15(55.6%)	0.755[c]
	≥ 5 year n(%)	16(48.5%)	12(44.4%)	
Use of NSAIDs		2(6.1%)	6(22.2%)	0.124[b]
Family Hx of AS		7(21.2%)	8(29.6%)	0.454[c]
Presence of	**Osteoporosis**	9(27.3%)	5(19.2%)	0.471[c]
	Uveitis	3(9.1%)	3(11.5%)	0.999[c]
	IBD	0(0.0%)	2(7.7%)	0.190[c]

Continuous variables presented as mean± SD; Discrete variables presented as numbers and frequencies;R=responder;NR=non-responder; n=number; BMI=body mass index with a unit of kilogram per square meter;Hx=history; IBD=inflammatory bowel disease;

[a]independent sample t-test;

[b]Fisher's exact test;

[c]chi-square test

*= highly significant difference between two groups.

Table 4. Clinical characteristics variables of the study groups.

Parameters	R (n=33)	NR (n=27)	p-value
TNF-α (pg/ml)	92.6±35.2	135.3±37.0	<0.001*[a]
hs-CRP (mg/l)	3.4±3.0	8.5±4.0	<0.001*[a]
Baseline WBC (10^3/μl)	8.6±2.5	8.0±2.9	0.363[a]
WBC after treatment (10^3/μl)	7.7±2.0	7.4±1.8	0.512[a]
Baseline ESR (mm/h)	30.1±19.7	38.1±16.8	0.104[a]
ESR after treatment (mm/h)	16.8±10.9	31.2±17.5	<0.001*[a]
Baseline BASDAI	4.4±0.7	4.9±1.3	0.115[a]
BASDAI after treatment	2.2±0.8	4.8±1.1	<0.001*[a]
BASFAI after treatment	2.5±1.3	5.0±1.4	<0.001*[a]

Continuous variables presented as mean± SD; R=responder; NR=non-responder; WBC=white blood cell; BASDAI=Bath Ankylosing Spondylitis Disease Activity Index; BASFI=Bath Ankylosing Spondylitis Functional Index. * highly significant difference between two groups; [a]: independent sample t-test.

Table 5. Frequency of alleles and genotypes of (-1031T/C), (-857C/T), (-806C/T) TNF-α gene in ankylosing spondylitis patients (n=60).

SNPs	Genotypes	n	%
rs1799964	TT	44	73.3
	TC	15	25
	CC	1	1.7
Allele frequency	T	103	85.8
	C	17	14.2
rs4248158	CC	55	91.7
	CT	5	8.3
Allele frequency	C	115	95.8
	T	5	4.2
rs1799724	CC	38	63.3
	CT	22	36.7
Allele frequency	C	98	81.7
	T	22	18.3

neutrophils, mucosal-associated invariant T cells, and mast cells, at disease-prone sites[23].

The first biological DMARDs considered second-line therapy are TNFi. Adalimumab, certolizumab pegol, ETN, golimumab, and infliximab, as well as its biosimilars, are now indicated. Anti-TNF medication is quite successful in AS, with significant improvements in disease activity and functional scores following six months of treatment[24]. These drugs should be used according to their indications, contraindications, and patient comorbidities. No TNFi are recommended for preferred efficacy[25]. In large, randomized, controlled clinical trials, TNF-α inhibitors have shown efficacy and are well-tolerated. However, a considerable minority of individuals do not respond to these medications, and their use may be associated with severe adverse drug reactions.

It is worth mentioning that the most reported adverse drug reactions to biological drugs in a study performed in Iraq were related to general disorders and administration site conditions, followed by skin and subcutaneous disorders, respiratory disorders, and gastrointestinal disorders[26]. In the current research, the mean age in the responder and non-responder groups was not significantly different ($p = 0.559$). The study results revealed that there was a significant difference ($p < 0.005$) in gender distribution, in which the percentage of males in the responder group was 97%, whereas it was 70.4% in the non-responder group, while the percentage of females in the responder and non-responder groups was 3% and 29.6%, respectively; this result is consistent with various previous studies[27–29].

The serum TNF-α and high-sensitivity C-reactive protein (hs-CRP) levels were also shown to vary significantly between the responder and non-responder groups. The mean BASDAI (4.8 ± 1.1) and BASFI) 5.0 ± 1.4) of the non-responder group treated with ETN was higher than the responder group, and this difference was statistically significant (p-value < 0.001). Additionally, the mean serum level of ESR after treatment

Table 6. Distribution of TNF-α gene polymorphism and individual alleles in the AS patient groups.

SNPs	Genotypes	Responder group (n=33) N(%)	Non-responder group (n=27) N (%)	p-value
rs1799964T/C	TT	28 (84.8)	16 (59.3)	0.02*
	TC	4 (12.1)	11 (40.7)	0.01*
	CC	1 (3.0)	0 (0.0)	1.00
Allele frequency	T	60 (90.9)	43 (79.6)	0.07
	C	6 (9.1)	11 (20.4)	
rs1799724C/T	CC	20 (60.6)	18 (66.7)	0.62
	CT	13 (39.4)	9 (33.3)	
Allele frequency	C	53 (80.3)	45 (83.3)	0.66
	T	13 (19.7)	9 (16.7)	0.06
rs4248158C/T	CC	31 (93.9)	24 (88.9)	0.48
	CT	2 (6.1)	3 (11.1)	0.64
Allele frequency	C	64 (97.0)	51 (94.4)	0.49
	T	2 (3.03)	3 (5.6)	0.65

* statistically significant difference between two groups.

Table 7. Binary logistic regression analysis of genotypes to predict the tendency of being a responder to ETN.

SNPs	OR	95% CI		p-value
		Lower	Upper	
rs1799964				
TC	0.208	0.057	0.762	0.018*
CC	NA	.....	.....	1.000
rs1799724				
CT	1.204	0.404	3.586	0.739
rs4248158		0.082	3.774	0.548
CT	0.556			

* significant strength of association; OR: odds ratio; CI: confidence interval; p: significance level; NA: non-applicable.

Reference level for each SNP is the wild allele: TT for rs1799964T/C; CC for rs1799724C/T; CC for rs428158C/T.

Table 8. Relationship between each genotype and the probability of being a responder.

SNPs		Phi-coefficient	P-value
rs1799964T/C	TT	0.288	0.025*
	TC	−0.329	0.011*
	CC	0.118	0.362
rs1799724C/T	CC	−0.063	0.628
	CT	0.063	0.628
Rs4248158C/T	CC	0.091	0.481
	CT	−0.091	0.481

* statistically significant

with ETN was higher in the non-responder group (31.2 ± 17.5) than the responder group (16.8 ± 10.9), and this difference was statistically significant (p-value < 0.001). This finding is close to the result of a previously performed study by Albagoa *et al.*[30].

Prior studies conducted in Iraq examined the association between genetic variants in different genes and the prevalence and severity of AS[31–33]. This observational study is the first study in Iraq that has analyzed the association of SNPs in the TNF-α promoter region gene -1031T/C (rs1799964), -857C/T (rs1799724) and -806C/T (rs 4248158) with outcomes of treatment in 60 Iraqi patients with AS taking ETN.

Numerous global researchers have investigated the influence of SNPs in the different genes on the TNFi response[34–37]. Regarding the frequency of the prevalence of -1031T/C (rs1799964) in all AS patients, the present study showed that the wild TT homozygote in rs1799964T/C was more frequent and predominant in over half of patients (73%), followed by heterozygote TC (25%) and a low proportion of mutant CC homozygote

Table 9. Distribution of studied patients according to the occurrence of infection, injection site reaction, headache, and rash as a side effect and encountered alleles.

SNPs	Genotypes	Infection		P-value
		Yes N (%), n=17	No N (%), n=43	
rs1799964T/C	TT	13 (76.4)	31 (72.0)	1.00
	TC	4 (28.5)	11 (25.5)	
	CC	0 (0.0)	1 (2.3)	
rs1799724C/T	CC	10 (58.8)	28 (65.1)	0.649
	CT	7 (41.2)	15 (34.9)	
rs 4248158C/T	CC	15 (88.2)	40 (93.0)	0.545
	CT	2 (11.8)	3 (7.0)	
SNPs	**Genotypes**	**Injection site reaction**		**P-value**
		Yes N (%), n=21	**No N (%), n=39**	
rs1799964T/C	TT	17 (80.9)	27 (69.2)	0.376
	TC	4 (19.0)	11 (28.2)	
	CC	0 (0.0)	1 (2.5)	
rs1799724C/T	CC	14 (66.7)	24 (61.5)	0.694
	CT	7 (33.3)	15 (38.5)	
rs 4248158C/T	CC	21 (100.0)	34 (87.2)	0.087
	CT	0 (0.0)	5 (12.8)	
SNPs	**Genotypes**	**Headache**		**P-value**
		Yes N (%), n=11	**No N (%), n=49**	
rs1799964T/C	TT	8 (72.72)	36 (73.46)	1.00
	TC	3 (27.27)	12 (24.48)	
	CC	0 (0.0)	1 (2.04)	
rs1799724C/T	CC	8 (72.7)	30 (61.2)	0.474
	CT	3 (27.3)	19 (38.8)	
rs 4248158C/T	CC	11 (100.0)	44 (89.8)	0.278
	CT	0 (0.0)	5 (10.2)	
SNPs	**Genotypes**	**Rash**		**P-value**
		Yes N (%), n=12	**No N (%), n=48**	
rs1799964T/C	TT	10 (83.3)	34 (70.8)	0.485
	TC	2 (16.6)	13 (27.0)	
	CC	0 (0.0)	1 (2.0)	
rs1799724C/T	CC	6 (50.0)	32 (66.7)	0.284
	CT	6 (50.0)	16 (33.3)	
rs 4248158C/T	CC	12 (100.0)	43 (89.6)	0.243
	CT	0 (0.0)	5 (10.4)	

(2%). Moreover, the T allele was found in over 85% of patients, but the C allele was present in only about 14% of patients. There has been no previous research conducted in Iraq that closely examined -1031T/C on AS or any other disease to match it with; hence, the outcomes seemed to be equivalent to those of Han Chinese and Korean people with AS, demonstrated in previous research.

This result is consistent with the Sheng *et al.*[38] study, where the TT, TC, and CC genotypes were found in approximately 76%, 19%, and 4% of AS patients, respectively, with the proportion of T allele to C allele being 86 % and 14%, respectively. Similarly, Chung *et al.*[39]. showed a higher proportion of wild TT homozygote genotype in about 59 patients, followed by 18 patients with the TC heterozygote genotype and no patients with the CC genotype. Regarding the difference in -1031T/C (rs1799964) genotypes frequency between responders and non-responders, the analysis of this study showed a significant difference in the TT genotype of -1031T/C polymorphism (rs1799964)(p-value = 0.02) in the responder group, in contrast to the TC genotype, which was significantly more frequent in the non-responder group (p-value = 0.01), while there was no significant difference in the availability of the CC genotype between responder and non-responder groups. Similar findings were seen in a study by Tong *et al.*[40], who observed that the TT genotype had a better treatment outcome compared with the CT and CC genotypes.

The current research findings could be attributed to the fact that the availability of the -1031TT genotype diminished TNF-α concentrations in the serum. Regarding the frequency of the prevalence of -857 C/T (rs1799724) in all AS patients, the current data show that there was a high prevalence of CC genotype, occurring in more than half the patients. Besides that, the C allele was found in over 80% of patients, but the T allele was present in only about 18% of patients.

This result was in agreement with a study by Mohammed *et al.*[41], which covered 80 Iraqi RA patients and revealed the prevalence of the CC genotype, with the C allele found in 81.25%. In contrast, the T allele was found in only about 18% of participants. Similarly, an Italian study by Aita *et al.*[42] reported a high frequency of CC genotype (60%) compared with TC and TT genotypes in AS patients. In addition, the current investigation indicated that both CC and CT genotypes were distributed equally across study patients.

There was no significant difference statistically between the respondent and non-respondent groups, and without correlation with any response to ETN. The outcome seems to agree with the result of a previous study by Mohammed *et al.*[41]. However, the current findings disagree with the Chinese Han study by Tong *et al.*, which revealed that -857CC genotypes can predict positive prognosis responsiveness to TNF-α inhibitors[40]. Indeed, a meta-analysis of a variety of ethnic groupings highlighted a relationship between the -857 C allele and the sensitivity of TNF-inhibitors in Caucasians, which was not observed in Asians[35]. Put another way, many investigations indicate that TNF-α -857CC improves the responsiveness to treatment (ETN), despite contradictory and insignificant evidence for this relationship[43].

This disparity in results comparing the current study to earlier research can be associated with the rarity of the T allele in patients with AS in Iraq who participated in the current research and may be because of genetic heterogeneity and different environmental factors in different populations. In the instance of -806C/T genotypes, the results of this investigation demonstrated that the CC genotype was widespread, in more than 90% of patients, followed by the CT genotype in about 8% of participants. More than 95% of patients carried the C allele, while just 4% carried the T allele.

This is the first study to examine the genotyping of -806C/T in AS patients from Iraq. Nevertheless, the results were equivalent to those from investigational research on the hazards of intracranial aneurysms among Chinese people[44]. Furthermore, addressing the distinction in genotype frequency between the study groups, an analysis of this study showed a non-significant difference in CC and CT genotypes between the two groups. Similarly, while the CC genotype showed a positive relationship and the CT genotype showed a negative relationship, neither of them reached the statistical significance level. It is noteworthy that ours is the first study to investigate the association between the TNF-α -806C/T polymorphism and response to ETN in patients with AS. To investigate whether SNPs in the promoter region of the TNF-α gene alter the severity of harmful effects from ETN use, we looked at the relationship between genotype and the occurrence of these adverse events: infection, injection site reaction, headache, and rash.

These three SNPs were compared for each genotype between non-adverse and adverse events cases. The most frequently reported side effects were injection site reaction (21 patients, 35%), followed by infection (28.3%), then headache and rash, with an incidence of about 38%. The incidence of short-term and long-term adverse effects linked with TNF-blocker medication in Chinese Han patients with AS was investigated by Tong *et al.*[45], and they discovered that disease duration, ESR, and CRP levels in the serum were related to an increase in side effects when using TNF-blockers. Chronic therapy with infliximab correlated with more side effects than rhTNFR-Fc.

Moreover, Chou *et al.*[46] found no severe adverse events in a prospective, open-label trial of ETN, involving 46 patients with AS from 60 Taiwan medical centers. However, SNPs investigated in this study cannot effectively predict the occurrence of drug-related adverse events. There are significant limits to the generalizability of our study's findings. First, the limited sample size is a drawback of this study. Specifically, even though ETN is a highly effective treatment for AS, its expense and stringent inclusion criteria are frequently the primary reasons why published observations in AS patients, as well as our study, typically have a small sample size. Next, the present research was conducted at one institute, and, while our institute serves Iraqi people from many cities, we recommend that further studies may be necessary prior to expressing the results among all Iraqi AS individuals.. Lastly, a tiny proportion

of cases solely received ETN because of inadequate budget and resources. Regardless, it would be intriguing to increase the sample size to validate the result. Indeed, the small sample size and differences in genetic background amongst ethnic populations may explain the few contradictory findings.

Conclusion

The presence of the TT genotype of rs1799964 is associated with a higher likelihood of responding to ETN, suggesting that it is a useful diagnostic for predicting response in Iraqi AS patients. In contrast, the TC genotype of rs1799964 greatly increases the risk of becoming an ETN non-responder. These results suggest that the TC genotype of rs1799964 should be investigated in AS patients prior to delivery of ETN.

Ethical approval

Ethical approval was obtained from the Scientific and Ethical Committee at the College of Pharmacy, Baghdad University, Iraq (approval number: RECACPUB-3102020D). This research was conducted in line with the Helsinki Declaration[18].

Consent

Before data collection, written consent was obtained from each of the participants.

Data availability

Underlying data

Zenodo: Association between polymorphisms within gene coding for tumor necrosis factor (TNF)-alpha with outcomes of treatment in sample of Iraqi patients with Ankylosing Spondylitis taking Etanercept. https://doi.org/10.5281/zenodo.7339117[47].

This project contains the following underlying data:

- Article data.xlsx (Demographic data, disease characteristics, and laboratory findings.)

References

1. Kasper D, Fauci A, Hauser S, *et al.*: **Harrison's principles of internal medicine.** 19e: Mcgraw-hill New York, NY, USA; 2015.
Reference Source
2. Greene RJ, Harris ND: **Pathology and Therapeutics for Pharmacists**. Pharmaceutical Press; 2020.
Reference Source
3. Papadakis MA, McPhee SJ, Rabow MC: **Medical Diagnosis & Treatment**. Mc Graw Hill: San Francisco, CA, USA; 2019.
4. Braun J, Sieper J: **Ankylosing spondylitis.** *Lancet.* 2007; **369**(9570): 1379–90.
PubMed Abstract | Publisher Full Text
5. Dougados M, Baeten D: **Spondyloarthritis.** *Lancet.* 2011; **377**(9783): 2127–37.
PubMed Abstract | Publisher Full Text
6. Al-Bedri KZM: **Prevalence, clinical features, and radiological features of Iraqi patients with ankylosing spondylitis.** *J Nat Appl Sci.* 2014; **4**(24).
Reference Source
7. Mustafa KN, Hammoudeh M, Khan MA: **HLA-B27 prevalence in Arab populations and among patients with ankylosing spondylitis.** *J Rheumatol.* 2012; **39**(8): 1675–7.
PubMed Abstract | Publisher Full Text
8. Zambrano-Zaragoza JF, Agraz-Cibrian JM, González-Reyes C, *et al.*: **Ankylosing spondylitis: from cells to genes.** *Int J Inflam.* 2013; **2013**: 501653.
PubMed Abstract | Publisher Full Text | Free Full Text
9. Parnham MJ, Bruinvels J: **Milestones in Drug Therapy MDT**. 2006.
Reference Source
10. DiPiro JT, Talbert RL, Yee GC, *et al.*: **Pharmacotherapy: A Pathophysiologic Approach.** ed. Connecticut: Appleton and Lange, 2014; **4**: 141–2.
Reference Source
11. Baseggio L, Bartholin L, Chantome A, *et al.*: **Allele-specific binding to the -308 single nucleotide polymorphism site in the tumour necrosis factor-alpha promoter.** *Eur J Immunogenet.* 2004; **31**(1): 15–9.
PubMed Abstract | Publisher Full Text
12. Bergman MJ, Kivitz AJ, Pappas DA, *et al.*: **Clinical utility and cost savings in predicting inadequate response to anti-TNF therapies in rheumatoid arthritis.** *Rheumatol Ther.* 2020; **7**(4): 775–92.
PubMed Abstract | Publisher Full Text | Free Full Text
13. Ma MHY, Defranoux N, Li W, *et al.*: **A multi-biomarker disease activity score can predict sustained remission in rheumatoid arthritis.** *Arthritis Res Ther.* 2020; **22**(1): 158.
PubMed Abstract | Publisher Full Text | Free Full Text
14. Ventola CL: **Role of pharmacogenomic biomarkers in predicting and improving drug response: part 1: the clinical significance of pharmacogenetic variants.** *P T.* 2013; **38**(9): 545–60.
PubMed Abstract | Free Full Text
15. Rao M, Wong C, Kanetsky P, *et al.*: **Cytokine gene polymorphism and progression of renal and cardiovascular diseases.** *Kidney Int.* 2007; **72**(5): 549–56.
PubMed Abstract | Publisher Full Text
16. Wu X, Sheng X, Sheng R, *et al.*: **Genetic and clinical markers for predicting treatment responsiveness in rheumatoid arthritis.** *Front Med.* 2019; **13**(4): 411–9.
PubMed Abstract | Publisher Full Text
17. Prieto-Pérez R, Cabaleiro T, Daudén E, *et al.*: **Gene polymorphisms that can predict response to anti-TNF therapy in patients with psoriasis and related autoimmune diseases.** *Pharmacogenomics J.* 2013; **13**(4): 297–305.
PubMed Abstract | Publisher Full Text
18. General Assembly of the World Medical Association: **World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.** *J Am Coll Dent.* 2014; **81**(3): 14–8.
PubMed Abstract
19. van der Linden S, Valkenburg HA, Cats A: **Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria.** *Arthritis Rheum.* 1984; **27**(4): 361–8.
PubMed Abstract | Publisher Full Text
20. Rudwaleit M, Listing J, Brandt J, *et al.*: **Prediction of a major clinical response (BASDAI 50) to tumour necrosis factor α blockers in ankylosing spondylitis.** *Ann Rheum Dis.* 2004; **63**(6): 665–70.
PubMed Abstract | Publisher Full Text | Free Full Text
21. Markowitz JS: **Body mass index (BMI)**. Mortality and Its Risk Factors Among Professional Athletes: Springer; 2018; 39–49.
22. Joshi M, Deshpande JD: **Polymerase chain reaction: methods, principles and application.** *Int J Biomed Res.* 2010; **2**(1): 81–97.
Publisher Full Text
23. Mauro D, Thomas R, Guggino G, *et al.*: **Ankylosing spondylitis: an autoimmune or autoinflammatory disease?** *Nat Rev Rheumatol.* 2021; **17**(7): 387–404.
PubMed Abstract | Publisher Full Text
24. Hyrich KL, Watson KD, Silman AJ, *et al.*: **Predictors of response to anti-TNF-α therapy among patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Register.** *Rheumatology (Oxford).* 2006; **45**(12): 1558–65.
PubMed Abstract | Publisher Full Text
25. van der Heijde D, Ramiro S, Landewé R, *et al.*: **2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis.** *Ann Rheum Dis.*

2017; **76**(6): 978–91.
PubMed Abstract | Publisher Full Text

26. Hassan EF, Kadhim DJ, Younus MM: **Safety profile of biological drugs in clinical practice: a retrospective pharmacovigilance study.** *Iraqi Journal of Pharmaceutical Sciences.* 2022; **31**(1): 32–42.
Publisher Full Text

27. Al-Osami MH, Gorial FI, Albeer MR, *et al.*: **Etanercept is effective and relatively safe in a sample of Iraqi patients with ankylosing spondylitis.** *JNSR.* 2013; **3**(14): 124–30.
Reference Source

28. Al Hafidh AH: **Clinical and epidemiological aspects of ankylosing spondylitis patients in a single center in Baghdad.** *Journal of Techniques.* 2022; **4**(1): 62–6.
Reference Source

29. Al-Shaibani SAR, Jassim NA, Al-Bayati AAK: **Raised inflammatory markers as predictors of response to anti-tumor necrosis factor drugs (etanercept and infliximab) in a sample of Iraqi patients with ankylosing spondylitis.** *Medical Journal of Babylon.* 2021; **18**(3): 241–244.
Reference Source

30. Albagoa ZR, Thanoon IA, Abdulla FI, *et al.*: **Etanercept in patients with ankylosing spondylitis: effectiveness and rate of response.** *MMSL.* 2022; **91**(4): 266–273.
Publisher Full Text

31. Al-Taee MM, Jassim HM, Alosami MH: **Genetic polymorphism in endoplasmic reticulum aminopeptidase-l (*erap1*) gene in Iraqi patients with ankylosing spondylitis.** *World J Pharm Res.* 2016; **5**(6): 321–32.
Reference Source

32. Daekh NA, Mohammed KA, Ali NH: **Polymorphism of HLA-B27 among ankylosing spondylitis patients in Basrah, Iraq.** *Sci J Med Res.* 2020; **4**(13): 12–6.
Reference Source

33. Al-Tarboolee AYK, Al-Taee MM, Kadir OKAAL: **MHC Class I polypeptiderelated sequence A (MICA) polymorphism association with ankylosing spondylitis in Iraqi patients.** *Indian Journal of Forensic Medicine & Toxicology.* 2020; **50**(4): 356–361.
Reference Source

34. Reich K, Hüffmeier U, König IR, *et al.*: **TNF polymorphisms in psoriasis: association of psoriatic arthritis with the promoter polymorphism TNF*-857 independent of the PSORS1 risk allele.** *Arthritis Rheum.* 2007; **56**(6): 2056–64.
PubMed Abstract | Publisher Full Text

35. Song GG, Seo YH, Kim JH, *et al.*: **Association between *TNF*-α (-308 A/G, -238 A/G, -857 C/T) polymorphisms and responsiveness to TNF-α blockers in spondyloarthropathy, psoriasis and Crohn's disease: a meta-analysis.** *Pharmacogenomics.* 2015; **16**(12): 1427–37.
PubMed Abstract | Publisher Full Text

36. Tong Q, Zhao L, Qian XD, *et al.*: **Association of *TNF*-α polymorphism with prediction of response to TNF blockers in spondyloarthritis and inflammatory bowel disease: a meta-analysis.** *Pharmacogenomics.* 2013; **14**(14): 1691–700.
PubMed Abstract | Publisher Full Text

37. Fabris M, Quartuccio L, Fabro C, *et al.*: **The -308 TNFα and the -174 IL-6 promoter polymorphisms associate with effective anti-TNFα treatment in seronegative spondyloarthritis.** *Pharmacogenomics J.* 2016; **16**(3): 238–42.
PubMed Abstract | Publisher Full Text

38. Sheng N, Gao Y, Li H, *et al.*: **The associations of rs1799724 and rs361525 with the risk of ankylosing spondylitis are dependent on HLA-B27 status in a Chinese Han Population.** *Front Immunol.* 2022; **13**: 852326.
PubMed Abstract | Publisher Full Text | Free Full Text

39. Chung WT, Choe JY, Jang WC, *et al.*: **Polymorphisms of tumor necrosis factor-α promoter region for susceptibility to HLA-B27-positive ankylosing spondylitis in Korean population.** *Rheumatol Int.* 2011; **31**(9): 1167–75.
PubMed Abstract | Publisher Full Text

40. Tong Q, Zhao DB, Bajracharya P, *et al.*: ***TNF*-α -857 and -1031 polymorphisms predict good therapeutic response to TNF-α blockers in Chinese Han patients with ankylosing spondylitis.** *Pharmacogenomics.* 2012; **13**(13): 1459–67.
PubMed Abstract | Publisher Full Text

41. Mohammed S, Zalzala M, Gorial F: **Association of tumor necrosis factor-alpha promoter region gene polymorphism at positions -308G/A, -857C/T, and -863C/A with etanercept response in Iraqi rheumatoid arthritis patients.** *Arch Rheumatol.* 2022; **37**(4): 613–625.
Publisher Full Text

42. Aita A, Basso D, Ramonda R, *et al.*: **Genetics in TNF-TNFR pathway: A complex network causing spondyloarthritis and conditioning response to anti-TNFα therapy.** *PLoS One.* 2018; **13**(3): e0194693.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Murdaca G, Negrini S, Magnani O, *et al.*: **Impact of pharmacogenomics upon the therapeutic response to etanercept in psoriasis and psoriatic arthritis.** *Expert Opin Drug Saf.* 2017; **16**(10): 1173–9.
PubMed Abstract | Publisher Full Text

44. Hu J, Luo J, Wang H, *et al.*: **Association of TNF-α-3959T/C Gene Polymorphisms in the Chinese Population with Intracranial Aneurysms.** *J Mol Neurosci.* 2017; **63**(3–4): 349–54.
PubMed Abstract | Publisher Full Text

45. Tong Q, Cai Q, de Mooij T, *et al.*: **Adverse events of anti-tumor necrosis factor α therapy in ankylosing spondylitis.** *PLoS One.* 2015; **10**(3): e0119897.
PubMed Abstract | Publisher Full Text | Free Full Text

46. Chou CT, Tsai CY, Liang TH, *et al.*: **Better short-term clinical response to etanercept in Chinese than Caucasian patients with active ankylosing spondylitis.** *Mod Rheumatol.* 2010; **20**(6): 580–7.
PubMed Abstract | Publisher Full Text

47. Shareef LG: **Demographic data, disease characteristics, and laboratory findings for Association between polymorphisms within gene coding for tumor necrosis factor (TNF)-alpha with outcomes of treatment in sample of Iraqi patients with Ankylosing Spondylitis taking Etanercept.** 2022.
http://www.doi.org/10.5281/zenodo.7339117