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The Effect of Tumor Necrotic Factor Alpha Polymorphism on Response to Biological Treatment for Rheumatoid Arthritis Patients

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Abstract

Background The tumor necrotic factor alpha (TNF-α) was associated with rheumatoid arthritis (RA) pathogenesis and inflammation of joint, for this consider important target for drugs (TNF inhibitors) as for example infliximab.

Objective To investigate the role of TNF-α-308 polymorphism G>A in unresponsiveness to biological treatment (infliximab) in RA patients.

Methods This study included 29 RA patients treated with infliximab attending Department of Rheumatology in Baghdad Teaching Hospital and diagnosed according to American College of Rheumatology (ACR) with 30 persons as healthy controls. The age range was 20 to 68 years. The DAS28 was calculated for each RA patients. Blood samples were taken from them during the period from May 2014 to January 2015. The blood samples were tested by polymerase chain reaction (PCR) for TNF-α-308 G>A then by restriction fragment length polymorphism (RFLP) for A allele and G allele. HLA-DR genotyping by PCR-SSO.

Results The frequency of A allele was 23 (39.7%) in RA patients while G allele was 35 (60.3%) in RA patients, whereas in in controls A and G alleles frequencies were 5 (8.3%) and 55 (91.7%) respectively. The A allele was associated with high DAS28 19 (82.6% while for G allele was 1 (2.9%) and DAS28 showing significant association with TNF-α-308 polymorphism G>A. The distribution of A allele in female RA patients was 16 (36%) whereas G allele was 28(64%), while in male RA patients, A and G allele were 7 (50%) for each of them. The HLA-DRB1 not showing significant association with TNF-α-308 polymorphism G>A.

Conclusion The TNF-α-308 polymorphism G>A has effect on response to biological treatment (infliximab). The A allele of TNF-α was associated with unresponsiveness to infliximab and RA patients who carry this allele have high DAS28 score. The HLA-DRB1 not showing association with TNF-α-308 polymorphism G>A.

Keywords RA, TNF-α polymorphism, infliximab, HLA-DR.

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List of abbreviations: ACR = American college of rheumatology, DAS = Disease activity score, HLA = Human leukocyte antigen, RA = Rheumatoid arthritis, TNF = Tumor necrosis factor.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting 0.2-1% of the population worldwide and is associated with progressive destruction of the joints causes early mortality, disability and

compromised excellence of life in the industrialized and developing world. Assessment of disease activity and treatment responses is based on measurement of disease activity score (DAS) [1].

Tumor necrotic factor (TNF), which is produce by monocytes, macrophage, stimulated T-cell, NK cell, neutrophil and fibroblast play important role in pathogenesis of rheumatoid arthritis, which act by activating leukocytes, endothelial cells, and synovial fibroblasts, inducing production of cytokines, chemokines, adhesion molecules, and matrix enzymes; suppression of regulatory T-cell function; activation of osteoclasts; and resorption of cartilage and bone; mediates metabolic and cognitive dysfunction. Consequently, TNF was target for drugs to treatment RA including infliximab which act as TNF inhibitor [2].

Tumor necrotic factor alpha is considered one of important mediator in pathogenesis of RA, polymorphism in TNF-α have been associated with severity and susceptibility of RA to biological treatment. Promoter polymorphisms at TNF have been associated with disease susceptibility, or severity of joint damage and autoantibody production in RA indifferent populations [3].

Individuals with HLA DRB1 alleles *0101, *0401, *0404 and *0405 have a much greater relative risk developing RA. Genes – including those risk alleles within HLA-DR4 – have been implicated but are insufficient to explain the vast majority of cases [4].

This study aimed to investigate the role of TNF-α-308 polymorphism G>A in unresponsiveness to biological treatment (infliximab) in RA patients.

Methods

This study included 29 RA patients on biological treatment with 30 individuals of healthy controls. The RA patients attended Rheumatology Department in Baghdad Teaching Hospital was examined by rheumatologist and DAS28 was calculated for each patient according to American college of rheumatology (ACR).

Five ml of blood was taken from each patient and healthy control by venipuncture, then 3 ml was added in to EDTA tubes for TNF-α-308 polymorphism and HLA-DR genotyping while 1.6 ml was added to 0.4 of sodium citrate for ESR.

DNA extraction from blood samples

Reliaprep blood gDNA (Miniprep system) provided by promeqa/USA and extraction of DNA was done according to manufacture instructions manual.

PCR for TNF-α-308 G>A

Tumor necrosis factor alpha was done as proposed by Dalziel et al [5].

Amplification primer for 308 tumor necrosis factor polymorphism.

Forward primer: AGGCAATAGGTTTTGAGGGCCAT

Reverse ward primer: ACACTCCCCATCCTCCCTG CT

Restriction fragment length polymorphism was done on gel by addition NCOI enzymes to PCR product [5].

HLA-DR genotyping was done PCR-SSO according to procedure by Innolipacompany (Beljum).

The study was approved by the Ethical Committee of the College of Medicine, Al-Nahrain University.

Statistical analysis

Distribution of genotyping and frequency of alleles were compared by chi-sequare test. Significance was attributed to probability values P≤ 0.05. Computer SPSS and Microsoft excel program were used for determination of probability values.

Results

This study was showed mean of age for RA patients on biological treatment (infliximab) was 42.72 years while healthy controls mean age was 43.6 years. The age range was 26 to 68 years in RA patients on other hand the age range in controls were 18 to 65 years with non-significant P value (>0.05) as in table (1).

Table 1. Mean of age in RA patients on treatment with infliximab and control

Age (Years)	Study groups	
	Control	RA patients
Mean	43.6	42.72
Standard deviation	11.28	11.24
Median	43	40
Minimum	18	26
Maximum	65	68
P value	>0.05	

The current results showed that the GG genotype of TNF-α-308 polymorphism G>A was detected in 16 (55.20%) out of 29 patients on treatment with infliximab while in control was in 27 (90.0%) out of 30 persons. In addition, the frequency of AA was 10 (34.50%) out of 29 patients on treatment with infliximab while in controls was 2 (6.70%) as well as the frequency G/A genotypes was detected in 3 (10.30%) of patients while in controls were only 1 (3.30%) and shown significant difference (P value <0.001) (Table 2 and Figure 1).

Percentage positivity of A alleles in RA patients on treatment was 23(39.70%), while in controls was 5 (8.30%). The frequency of G allele was 35 (60.3%) while in controls was 55 (91.7%) as shown in table (3).

Table (4) showed that A allele of TNF-α has high DAS score frequencies (82.6%) with no good response to biological treatment (infliximab), on other hand the G allele showing high frequencies of the remission cases with good response to biological treatment(infliximab) with significant association between DAS and TNF-α (P value <0.001).

The frequency of G allele was 28 (64%) in RA females' patients while A allele was 16 (36%), however, A and G allele in RA males' patients was 50% for each allele but the association between TNF-α allele and gender not showing significant association (P value=0.532) as shown in table (5).

Table 2. The TNF-α-308 polymorphism genotyping in controls and treated RA patients

TNF-α Genotypes		Studied Groups		
		Control	RA patients on treatment	Total
GG	Count	27	16	43
	%	90.0%	55.2%	47.8%
G/A	Count	1	3	4
	%	3.3%	10.3%	4.4%
AA	Count	2	10	12
	%	6.7%	34.5%	13.3%
Total	Count	30	29	59
	%	100%	100%	100%
P Value		0.001		

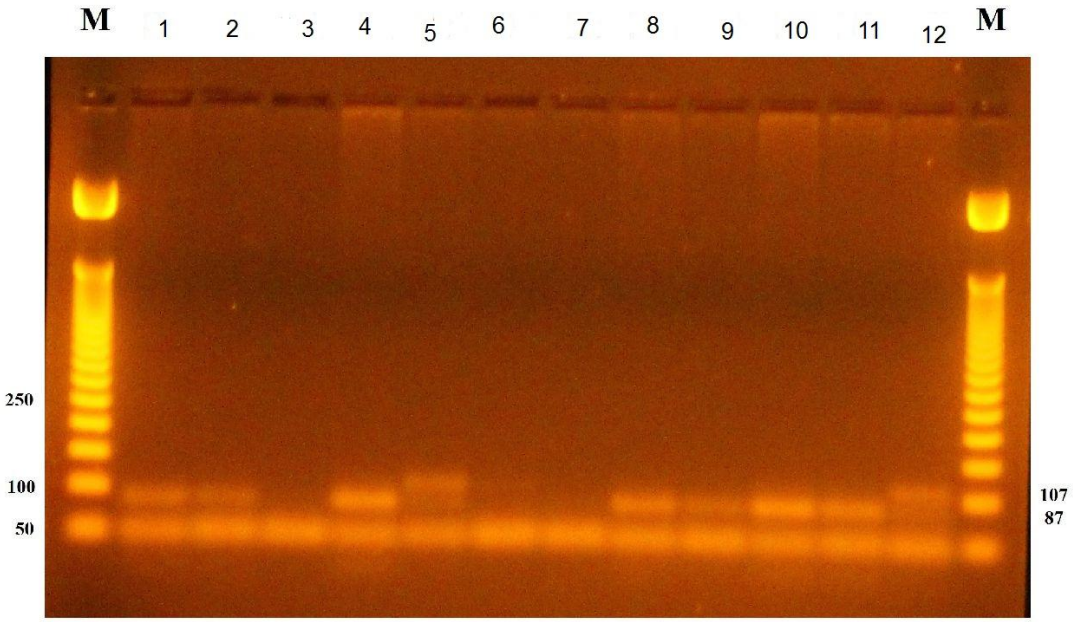

Figure 1. Gel electrophoresis for PCR product of TNF-α-308 G>A after digestion with restriction enzyme NCOI (107 bp was AA, 87 and 107 bp GA, 107 bp GG), M:1000 bp marker, (90 mint., 100 volt)

Table 3. The frequency of A and G alleles in RA patients on treatment

TNF-α-308 G>A		Studied Groups		
		Control	RA patients on treatment	Total
Allele A	Count	5	23	28
	%	8.3%	39.7%	15.6%
Allele G	Count	55	35	90
	%	91.7%	60.3%	85.4%
Total	Count	60	58	118
	%	100%	100%	100%

Table 4. Association between DAS score and TNF-α-308 G>A polymorphism in rheumatoid arthritis patients

DAS score	TNF-α-308 G>A			
	A	%	G	%
Remission	0	0.0%	12	34.3%
Mild	2	8.7%	10	28.6%
Moderate	2	8.7%	12	34.3%
High	19	82.6%	1	2.9%
Total	23	100%	35	100%
P value	<0.001			

Table 5. Association between TNF-α-308 G>A alleles and gender

Gender type	A	G	Total
Female	16	28	44
%	36%	64%	100%
Male	7	7	14
%	50%	50%	100%
Total	23	35	58
P value	0.532		
Odds ratio	0.57		
95% confidence interval	0.17 - 1.93		

Discussion

This study included 29 patients on treatment with infliximab, the wild genotyping GG was (55.20%) of RA patients on treatment while in control was (90%), this genotyping showing good response to biological treatment, controversy to genotyping AA and GA, Mugnier et al. (2003), mentioned that the GG genotyping represent wild genotyping and more common while AA and GA represent mutant genotyping [(6)]. Translation and posttranslation of TNF-α may causes 308 mutation in TNF-α, Wilson et al. (1995), mentioned that the A dimorphism with possible functional consequence (G to A transition at position -308 in the promoter/enhancer region) has been described for the TNF locus. TNF-α A allele associated with high TNF-α production in addition to increase susceptibility to infection and autoimmune diseases especially RA [(7)]. In some studies, 308 mutations have been attributed to geographical variation and cultural constraints resulting in higher endogamy therefore, the effect of inbreeding in Jammu and Kashmir population may lead to increase minor allele frequency in residents [(8)].

The frequency of A allele was high in RA patients on treatment, which showing weak responses to infliximab. The A allele associated with bad outcome of RA disease as well as high TNF-α production this result agrees with Maxwell et al. (2008) who revealed that the A allele frequencies were more than G allele in RA patients receiving biological treatment with poor response [(9)]. Marotte et al. (2006) who used in their study large size of samples from RA patients on biological treatment showed that the A alleles were more frequency in RA patients with inadequate response to drugs [(10)]. This study found that the G allele was more frequent in control than RA patients, this mean G may consider low risk allele for RA while A allele risk for RA as explained by Lee et al. (2007) who mentioned that the A allele consider risk for RA in Latin American [(11)].

Current study found that the patients who have A allele have high DAS score and showing bad response to infliximab as when compared with G allele this result also mentioned by Hajeer et al. (2003) who observed that RA patients with TNF-308 genotypes A/A or A/G show a poor response to infliximab treatment at 22 weeks [(12)]. They therefore, believe that TNF-genotyping in RA patients may be a useful tool for predicting efficacy of treatment with infliximab, however this finding not agree with Lee et al. (2010), who showed that the polymorphism of TNF-α have no effect on responses to infliximab in patients with RA [(13)]. The infliximab work better with normal G allele in tumor necrosis factor alpha while polymorphism with homozygote AA and heterozygote GA lead to less inhibition with tumor necrosis factor inhibitors and result in low outcome of biological treatment which is very costly as well as consider last choice for treatment of RA patients and patients

disabilities of RA disease development [14]. In the present study no significant association between HLA genotyping and A, G allele of TNF alpha in RA patients on treatment and this result agree with Lacki et al. (2000), who mentioned that the TNF-α-308 polymorphism not showing association with HLA-DR [15], but this result not consistent with Jimena Cuenca et al. (2001), who mentioned alteration in HLA allele may result in variation in TNF gene for this increasing susceptibility but depending on ethnicity [16]. In study by Braun et al. (1996), mentioned that the TNF-α A allele associated with high TNF-α production [17]. However, in this study the detection was made to association between TNF-α-308 polymorphism and HLA-DRB1 whereas in other study by Jawaheer et al. (2002), who mentioned that the association between TNF-α-308 polymorphism and HLA-DRB3 [18]. Finally, HLA-DRB1 may associated with high TNF-α production not with polymorphism (whether TNF-α was carried A or G allele), this agree with Padyukov et al. (2004) who hypothesis that the HLA-DRB1 associated with high TNF production [19].

This study concluded that TNF-α-308 polymorphism A associated with bad responses to infliximab while G allele associated with good response. HLA-DRB alleles not associated with TNF-α-308 polymorphism.

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Author contribution
Hachim: conducted the sampling, processing and working. Dr. Abbas: design of the work, data interpretation, drafting and critical revision of the article. Dr. Alosami: samples collection.

Conflict of interest
The authors declare no conflict of interest.

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