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Tumour necrosis factor gene polymorphisms in Egyptian patients with rheumatoid arthritis and their relation to disease activity and severity

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Abstract

Aim of the study: *The present case control study was conducted to assess the association of LTA 252 A>G, TNF-α 308 G>A, and TNF-α 1031 T>C gene polymorphisms with rheumatoid arthritis (RA), and their involvement in disease activity and severity.*

Material and methods: *A total of 70 Egyptians, including 35 RA patients and 35 healthy control individuals, were included in the study. The RA patients comprised 34 females and one male. Cases with RA were diagnosed by a rheumatologist and fulfilled the 2010 ACR/EULAR criteria. Modified disease activity score (DAS28) was used to assess disease activity. Van Der Heijde-modified Sharp score (vdHSS) was used to assess radiological changes for assessment of disease severity. PCR-RFLP was used to detect the association of LTA 252 A>G, TNF-α 308 G>A, and TNF-α 1031 T>C gene polymorphisms with RA.*

Results: *TNF-α 308 G allele and TNF-α 308 GG genotype were significantly higher in RA patients compared to healthy control subjects ($p = 0.04$ and $p = 0.001$, respectively). TNF-α 308 G allele and GG genotype were significantly higher in the RA non-remission group compared to the remission group ($p = 0.008$, $p < 0.001$). Patients with the TNF-α 308 AG genotype had higher mean of Sharp score compared to the patients with the GG and AA genotypes ($p = 0.007$). There was no significant association between LTA 252 A>G and TNF-α 1031 T>C gene polymorphisms and RA.*

Conclusions: *Our results suggest that TNF-α 308 G/A gene polymorphism is genetically associated with RA and involved in disease activity and severity in Egyptian patients.*

Key words: *rheumatoid arthritis, genetic susceptibility, TNF polymorphisms, polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP).*

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects 1% of the population worldwide [1]. It is a systemic disease that involves the joints, organs, and other areas of the body such as the skin, eyes, heart, lungs, kidney, and spleen [2, 3].

RA displays complex inheritance resulting from an intricate interplay between an individual's environmental and genetic background [4]. It has a multifactorial aetiology, including a wide spectrum of clinical manifestations, variability in disease, progression, severity, and response to therapies [5].

Increased expression of pro- and anti-inflammatory cytokines detected in the affected tissues and serum of RA patients clearly illustrates the role of cytokines in the aetiopathology of RA [6]. Tumour necrosis factor (TNF), a proinflammatory cytokine, has been shown to play a significant role in the pathogenesis of multiple autoimmune diseases including RA [6-8].

TNF-α and lymphotoxin alpha (LTA) are closely related cytokines that share 30% of amino acid residues and have the same cell surface receptor [9]. Nedwin *et al.* [10] reported that the TNF-α and LTA genes are positioned in tandem on chromosome 6 between the class I and class II cluster of the major histocompatibility complex (chromosome 6p21.1-6p21.3). The proinflammatory cytokine TNF-α is one of the cardinal factors involved in RA inflammatory state [7]. TNF-α pleiotropic biological activities are mediated binding to TNF receptors (TNFR) type I and II [11].

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TNF-α plays a pivotal role in inflammation by inducing the expression of other proinflammatory molecules, chemotactic cytokines, and adhesion factors [12-14]. *In vivo* and *in vitro* studies have illustrated that high levels of TNF-α lead to exacerbation of the inflammatory response. This, together with its strong immunomodulatory activities, has been suggested to be important in the pathogenesis of various diseases such as asthma, systemic lupus erythematosus (SLE), and RA [7, 15, 16].

Amongst the five polymorphisms (at positions +691, -238, -308, -851, and -857) of *TNF-α 308* gene, which were detected by PCR-single strand conformation polymorphism (PCR-SSCP) analysis, TNF-α-308 polymorphism has been reported to be associated with autoimmune diseases including RA [14, 17]. The genetic variation on position -308 resulted in two allelic forms in which the existence of guanine (G) defines the common variant, and the existence of adenine (A) determines the less common one. LTA, which is linked closely to TNF-α, has also been shown to contribute to the susceptibility of multiple autoimmune diseases [18, 19]. A polymorphism has been detected at position +252 within the first intron of the TNF-β gene, consisting of a G (LTA +252 G) on one allele and an A (LTA +252 A) on the alternate allele. The presence of G at this position determined the mutant allele known as LTA*1 (allele-1), which is the less frequent allele in white subjects and is associated with increased TNF-α and LTA production [20, 21].

A study of a Japanese population has indicated that LTA (+252) polymorphism together with HLADRB1*0405 may have an influence on the predisposition to RA [22]. To the best of our knowledge there are only two cohorts elucidating the role of *TNF-α 308 G/A* polymorphism in RA patients in Egypt. On the other hand, there are no reports investigating the roles of *TNF-α 1031 T>C* and *LTA 252 A>G* gene polymorphisms among Egyptian RA patients. Given the known importance of TNF gene in inflammatory and/or immune functions and the variation in susceptibility to immune disorders in different ethnic groups, we investigated the possible association between *LTA 252 A>G*, *TNF-α 308 G>A*, and *TNF-α 1031 T>C* polymorphisms and susceptibility to RA in Egyptian patients. In addition, we investigated the association of these polymorphisms with disease activity and severity.

Material and methods

Patients

A total of 70 Egyptian persons, including 35 cases and 35 control subjects, were included in the study. The RA patients comprised 34 females and one male. The RA patients were recruited from the outpatient clinic of the Rheumatology and Rehabilitation Department of Beni Suef University Hospital, Egypt, between March 2014 and January 2015. Cases with RA were diagnosed by a rheumatologist and fulfilled the 2010 ACR/EULAR criteria for diagnosis of RA [23]. The healthy control subjects were unrelated Egyptian age- and sex-matched individuals who had no family history of autoimmune diseases. The control group lived in the same geographical area and had the same ethnic origin as the patients. All cases and control subjects were informed of the purpose of the study, and their consent was obtained. The local Ethics Committee approved the study.

Clinical and laboratory assessment

Blood samples were obtained from all patients for determination of erythrocyte sedimentation rate (ESR; Westergreen), C-reactive protein (CRP), and rheumatoid factor (RF) by semi-quantitative latex (AVITEX® CRP and AVITEX® RF, Omega Diagnostics). CRP was considered positive when > 6 mg/l while RF wass considered positive at ≥ 8 IU/ml. Anti-cyclic citrullinated peptide (anti-CCP) was determined using enzyme-linked immunosorbent technique (ELISA) using QUANTA Lite® CCP3IgG ELISA, INOVA Diagnostics. According to the manufacturer's protocol, serum was considered positive when the reading was ≥ 20 units.

The modified disease activity score DAS28 was calculated for all patients [24]. DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity. A patient is considered in remission if the DAS28 score is lower than 2.6.

In all patients, plain radiographs of both hands and feet in the posteroanterior projection were obtained. Van Der Heijde-modified Sharp score (vdHSS) was used to assess radiological changes [25].

Genotyping

Genomic DNA was extracted from EDTA anti-coagulated whole blood using QIAamp DNA Mini Kit (Cat. no. 51104, QIAGEN) according to the manufacturer's protocol. The three sequences flanking TNF-α 1031 T>C, TNF-α 308G>A, and LTA 252 A>G single nucleotide polymorphisms were amplified by polymerase chain reaction (PCR). Genotyping of these polymorphisms was determined by a restriction fragment length polymorphism (RFLP) assay [26].

The 270-bp region of the *TNF-α 1031* gene, encompassing the 1031T/C polymorphism site, was amplified via polymerase chain reaction (PCR) using the sense (5'-GGGGAGAACAAAAGGATAAG) and antisense (5'-CCCCATACTCGACTTTCATA) primer pair [27]. The total reaction volume was 25 µl: 5 µl DNA, 12.5 µl Dream Taq green PCR master mix (Fermentas), 1 µl of each primer and 5.5 µl nuclease-free water. Initially, the PCR reaction was subjected to denaturation for 5 min at 95°C, followed by 30 cycles of amplification (30 s at 95°C, 30 s at 55°C, and 30 s at 72°C). A final elongation step

(5 min at 72°C) was applied at the end of the 30 cycles [28]. The PCR is followed by digestion with the restriction enzyme BbsI (Thermo Scientific Cat. no. FDo574) according to the manufacturer's protocol (C allele, 159 and 111 bp; T allele, 270 bp) (Fig. 1) [28]. Digested PCR fragments were separated by 4% agarose gel electrophoresis stained with ethidium bromide followed by ultraviolet visualisation.

The primers (5'-AGGCAATAGGTTTTGAGGGC-CAT-3') and (5'TCCTCCCTGCTCCGATTCC G-3') were used to amplify the 107-bp DNA fragment of the TNF-α 308 G>A polymorphism. The total PCR reaction mixture was 25 µl: 5 µl DNA, 12.5 µl Dream Taq green PCR master mix (Fermentas), 1 µl of each primer and 5.5 µl nuclease-free water. The PCR Cycling conditions for TNF-α 308 G>A were performed according to the protocol described by Bonyadi *et al.*: 5 min for initial denaturation at 95°C; 35 cycles at 95°C for 1 min for denaturation, 30 s at 65°C for annealing and 30 s at 72°C for extension, followed by 5 min at 72°C for final extension [28]. After amplification, PCR products were digested (at 37°C) by NcoI (Themo Scientific Cat. no. FD0574) according to the manufacturer's protocol (G allele, 87 and 20 bp; A allele, 107 bp) (Fig. 2) [28]. Digested PCR products were electrophoresed in 4% agarose gel stained with ethidium bromide and followed by ultraviolet visualisation. Primers (5'-CCGTGCTTCGTGCTTTGGACTA-3') and (5'AGAGCTGGTGGGGACATGT CT G-3') were used to amplify the 740-bp DNA fragment to genotype the LTA 252 A>G polymorphism. The total PCR reaction mixture was 25 µl: 5 µl DNA, 12.5 µl Dream Taq green PCR master mix (Fermentas), 1 µl of each primer and 5.5 µl nuclease-free water. The PCR cycling conditions were performed according to the protocol described by Cabrara *et al.* [29] with a slight modification: initial denaturation at 95°C for 5 minutes followed by 35 cycles of amplification: 94°C for 45 sec., 62.5°C for 45 sec, and a final extension at 72°C for 1.5 min. PCR products were digested (at 37°C) by NcoI (Thermo Scientific Cat. no. FD0574) according to the manufacturer's protocol. Digested PCR products were electrophoresed in 4% agarose gel stained with ethidium bromide and followed by ultraviolet visualisation. The digested products generated 185 bp and 555 bp bands for the G allele and a 107 bp band for the A allele (Fig. 3) [29].

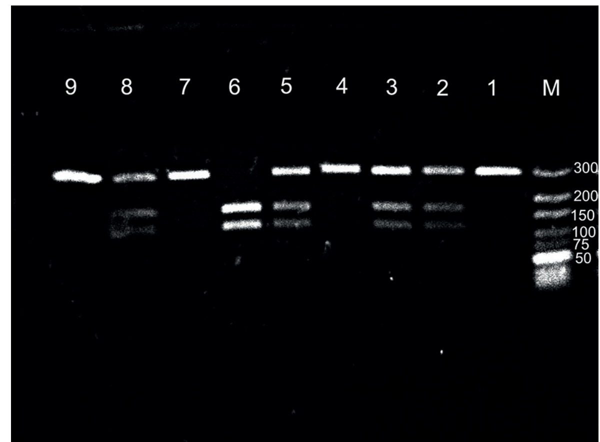

Fig. 1. PCR-RFLP analysis of TNF 1031 gene polymorphism using BbsI restriction enzyme. M: DNA molecular weight marker: (50 bp, 75 bp, 100 bp, 150 bp, 200 bp, 300 bp), lanes 1, 4, 7, 9: wild type (TT): 1 band at 270 bp, lane 6: homozygous mutant type (CC): 2 bands at 111 and 159 bp, lanes 2, 3, 5, 8: heterozygous mutant type (TC): 3 bands at 111 bp, 159 bp, and 270 bp

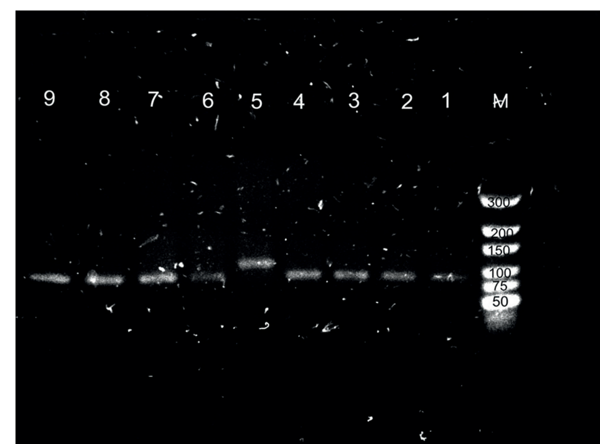

Fig. 2. PCR-RFLP analysis of TNF 308 gene polymorphism using NcoI restriction enzyme. M: DNA molecular weight marker: (50 bp, 75 bp, 100 bp, 150 bp, 200 bp, 300 bp), lanes 1, 2, 3, 4, 6, 7, 8, 9: wild type (GG): 2 bands at 87 bp and 20 bp, lane 5: homozygous mutant type (AA): 1 band at 107 bp

Statistical analysis

The collected data review, coding, and statistical analysis was done using SPSS software (Statistical Package for Social Science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows. Mean, median, range, and standard deviation were calculated to measure central tendency and dispersion of quantitative data, while the frequency of occurrence was calculated to measure qualitative data. Student's *t*-test was used to determine the significance of the difference between two means, χ^2 was carried out for comparison of qualitative data, and Fisher's exact test was used when the cell count was less than 5. Odds ratios (ORs) with 95% confidence intervals (CI) were calculated whenever applicable, to test the association between genotype and RA. Analysis of variance (ANOVA) test was used to determine the difference between more than two means. The significance of the OR was calculated using a 2 × 2 contingency table. Genotype distributions were compared with those expected for samples from populations in

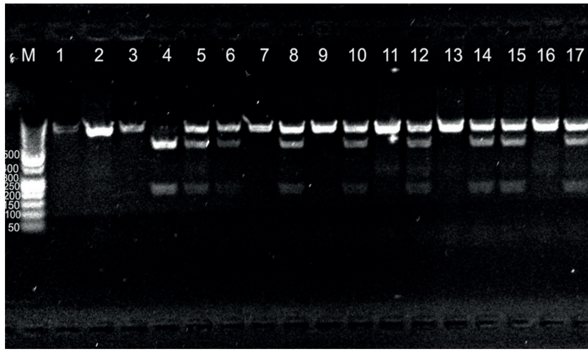

Fig. 3. PCR-RFLP analysis of LTA 252 gene polymorphism using NcoI restriction enzyme. M: DNA molecular weight marker: (50 bp, 100 bp, 150 bp, 200 bp, …, 500 bp), lanes 1, 2, 3, 7, 9, 11, 13, 16: wild type (AA): 1 band at 750 bp, lanes 5, 6, 8, 10, 12, 14, 15, 17: heterozygous mutant type (AG): 3 bands at 185 bp, 555 bp, and 750 bp, lane 4: homozygous mutant type (GG): 2 bands at 185 bp and 555 bp

Hardy-Weinberg equilibrium using an χ^2 test (1 df). The level of significance was taken at a p-value of < 0.05.

Results

The demographic, clinical, and laboratory data of RA patients and control group are shown in Table 1.

Concerning *LTA 252 A>G* gene polymorphism, the genotype frequencies of RA patients and healthy controls conformed to the Hardy-Weinberg equilibrium ($p = 0.2531$ and $p = 0.224$, respectively). Similarly, for the *TNF-α 1031 T>C* gene polymorphism, the genotype frequencies of RA patients and healthy controls conformed to the Hardy-Weinberg equilibrium ($p = 0.97$; $p = 0.13$, respectively). As regards the *TNF-α 308A>G* gene polymorphism, the genotype frequencies for cases showed deviation from HWE ($p = 0.0019$), while the genotype frequencies of controls conformed to HWE ($p = 0.08$) (Table 2).

Analysis of the distribution of TNF-α 308 G/A revealed that the frequency of the G allele was higher than the A allele in the non- remission group. On the other hand, only two patients carrying the A allele were in remission, and there were no patients in remission carrying the A allele. The difference between the two groups was statistically significant ($p = 0.008$). On comparing the distribution TNF-α 308 genotypes in both remission and non-remission group patients, the GG genotype was more frequently represented than the AG genotype and the AA genotypes, respectively, and the difference was highly statistically significant ($p < 0.001$) (Table 3). Analysis showed that the frequency of distribution of alleles and genotypes in TNF-α 1031 T/C and +252A/G polymorphism was statistically non-significant (Table 3).

On comparing the frequency of distribution of TNF-α 308 genotypes with disease severity, we detected that the patients with heterozygous mutant type (AG) had higher Sharp score than the patients with the wild type (GG) genotype and the homozygous mutant (AA) genotype, respectively, and the difference was statistically significant ($p = 0.007$) (Table 4). On the other hand, analysis of the frequency of distribution of genotypes in TNF-α 1031 and LTA +252 polymorphism according to disease severity was statistically non-significant ($p = 0.105$ and $p = 0.691$, respectively) (Table 4).

We did not detect any association between the distribution of TNF-α 308, TNF-α 1031, and LTA252 genotypes and age/sex of patients disease duration, CRP, RF, and anti-CCP positivity (data not shown).

Table 1. Demographic, clinical, and laboratory data of rheumatoid arthritis patients and control group

Demographic and clinical data	Cases (n = 35)	Controls (n = 35)	Significance test	p-value
Age			$t = 1.60$	0.112
Mean ±SD	45.63 ±13.93	40.51 ±12.63		
Range	20-70	22-65		
Sex, n (%)			$\chi^2 = 0.348$	0.555
Female	34 (97.1)	33 (94.3)		
Male	1 (2.9)	2 (5.7)		
Disease duration (year)				
Mean ±SD	8.49 ±6.95			
Median	6			
Range	1-30			
Sharp score				
Mean ±SD	37.31 ±26.40			
Median	30			
Range	10-90			
DAS28				
Mean ±SD	4.77 ±1.44			
Range	2.30-7.75			
ESR				
Mean ±SD	56.40 ±22.74			
Range	10-100			
CRP positivity	17/35 (48.6%)			
RF positivity	25/35 (71.4%)			
Anti-CCP positivity	29/35 (82.9%)			

Significant difference (p-value < 0.05), DAS – disease activity score, ESR – erythrocyte sedimentation rate, CRP – C-reactive protein, RF – rheumatoid factor, anti-CCP – anti-cyclic citrullinated peptide

Discussion

RA is a complex, multifactorial, inflammatory disease of unknown aetiology with considerable variability. Both

Table 2. Comparison of TNF-α and TNF-β polymorphisms between Egyptian rheumatoid arthritis patients and healthy controls

	Cases (*n* = 35)	Controls (*n* = 35)	Odds ratio and 95% confidence interval	χ^2	*p*-value
TNF-α 308, *n* (%)					
GG	30 (85.7)	19 (54.3)	Reference		< 0.001*
AG	3 (8.6)	16 (45.7)	8.42 (1.9- 42.3)	11.32	0.266
AA	2 (5.7)	0 (0.0)	0-0 (0.0-7.18)	1.24	
A	7 (10)	16 (22.86)	Reference	4.21	0.040*
G	63 (90)	54 (77.14)	2.67 (0.94-7.791)		
TNF-α 1031, *n* (%)					
TT	24 (68.5)	21 (60.0)	Reference		
TC	10 (28.6)	14 (40.0)	0.63 (0.20-1.90)	0.85	0.355
CC	1 (2.9)	0 (0.0)	NA	0.86	0.354
T	58 (82.86)	56 (80.0)	Reference	0.189	0.664
C	12 (17.14)	14 (20.0)	1.21 (0.47-3.084)		
LTA 252, *n* (%)					
AA	15 (42.9)	18 (51.4)	Reference		
AG	18 (51.4)	16 (45.7)	1.35 (0.46-3.95)	0.38	0.540
GG	2 (5.7)	1 (2.9)	2.40 (0.15-74.6)	0.50	0.481
A	48 (68.57)	52 (74.29)	Reference	0.56	0.454
G	22 (31.43)	18 (25.71)	0.76 (0.33-1.67)		

Significant difference (p-value < 0.05), allele frequency was calculated according to Hardy-Weinberg equation (HWE), NA – non-applicable

environmental and genetic factors have been related to susceptibility to disease initiation as well as outcome of disease course [30]. Fifty per cent of risk of developing RA is attributable to genetic factors [31]. Huge progress has been made in the detection of genetic regions that are characterised by structural variation (single nucleotide polymorphisms); more than 30 genetic regions are associated with the incidence of the occurrence of RA [32].

Single nucleotide polymorphisms are considerable and spread throughout the genome. Such disparities are associated with diversity in the population, susceptibility to diseases, and differential response to medical treatment [33].

Polymorphisms situated within the promoter region of TNF-α and the intron 1 polymorphism of LTA, in particular, have been linked with altered levels of circulating TNF-α [34].

In the present study, on investigating the genetic association of TNF-α 308 A/G and RA, the G allele and the wild type (GG) genotype were more frequently represented among RA patients ($p = 0.040$, $p < 0.001$; respectively). In accordance with our results, Mosaad *et al.* reported that the G allele and the GG genotype were more frequently represented among RA patients compared to the healthy control group [35]. Moreover, several studies conducted in other parts of the world are in agreement with our results [36, 37].

However, in contrast to the results of the current study, Hussein *et al.* [33] reported that TNF 308 AA genotype was more frequently prevalent among the patients and associated with RA susceptibility. Similarly, in contrast to our findings, numerous studies showed significantly higher frequency of allele A or genotype AA in RA patients compared to the control group, suggesting that TNF-α 308 A allele is a predisposing factor of RA [38-40]. On the other hand, non-significant association between TNF-α 308 polymorphism and RA susceptibility was observed in other case control studies [41, 42].

Our results regarding TNF-α 308 G/A polymorphism could be explained by the fact that these differences in findings may be attributed to the ethnicity-related genetic constitution in different populations, which is evident from the significant variability in genotype data of TNF-α 308 polymorphism among the healthy subjects of various ethnicities [37]. Hence, the joint gene-environment synergy might be responsible for the considerable differences in the results of polymorphism association studies on RA patients from diversities in ethnicities and/or geographical locations [42]. The difference in results between patient groups in different

Table 3. Comparison of TNF-α and TNF-β polymorphisms distribution according to DAS28 scoring system

DAS28	Remission (*n* = 1)	Non-remission (*n* = 34)	χ^2	*p*-value
TNF-α 308, *n* (%)				
GG	0 (0.0)	30 (88.2)	16.98	< 0.001*
AG	0 (0.0)	3 (8.9)		
AA	1 (100)	1 (2.9)		
A	2 (100)	5 (7.4)	18.53	0.008*
G	0 (0.0)	63 (92.6)		
TNF-α, *n* (%)				
CC	0 (0.0)	1 (2.9)	0.47	0.790
TC	0 (0.0)	10 (29.4)		
TT	1 (100)	23 (67.7)		
C	0 (0.0)	12 (17.6)	0.43	0.513
T	2 (100)	56 (82.4)		
LTA 252, *n* (%)				
GG	0 (0.0)	2 (5.9)	0.97	0.615
AG	1 (100)	17 (50.0)		
AA	0 (0.0)	15 (44.1)		
G	1 (50.0)	21 (30.9)	0.33	0.532
A	1 (50.0)	47 (79.1)		

Significant difference (p-value < 0.05), remission group – DAS28 < 2.6, nonremission group includes: low-activity patients (DAS28 from ≥ 2.6 to < 3.2), moderate-activity patients (DAS28 from ≥ 3.2 to ≤ 5.2), and high-activity group (DAS28 > 5.1), allele frequency was calculated according to HWE

Table 4. Comparison between disease severity (represented by Sharp score) and gene polymorphisms in RA patients

Genotypes	Sharp score (mean ±SD)	ANOVA test (*F*)	*p*-value
TNF-α 308		5.79	0.007*
GG	34.13 ±24.24		
AG	80.00 ±10.00		
AA	21.00 ±12.72		
TNF-α 1031		2.42	0.105
CC	90.00 ±12.01		
CT	31.50 ±24.86		
TT	37.54 ±21.18		
LTA 252		0.691	0.37
GG	37.50 ±24.74		
GA	41.00 ±28.67		
AA	32.87 ±24.75		

Significant difference (p < 0.05), the total number of cases analysed for each gene is 35

Egyptian governorates might be attributed to differences in study design, mean duration of RA, and sample size.

In the current study, allele and genotype frequency distribution analysis revealed that TNF-α 1031 T/C SNP is not associated with the incidence of RA in our study group. In agreement, Karry *et al.* found no significant difference between RA patients and healthy controls [27].

Although there is a plethora of literature regarding the role of TNF-α polymorphism in the pathogenesis of a variety of autoimmune diseases as RA, only a few studies have reported on the role of LTA in the pathogenesis of RA.

LTA belonging to the surrounding of TNF-α locus has indeed been shown to play a significant role in the pathogenesis multiple autoimmune diseases, including RA. Immunological studies on LTA showed its close similarity to TNF-α in terms of their pro-inflammatory and apoptotic activity [18].

A polymorphism has been detected at position +252 residing within the first intron of the LTA gene, consisting of nucleotides guanine (LTA+252G) on one allele and adenine (LTA +252A) on the alternate allele [37].

In the current study, LTA 252 A>G allele and genotype frequency distribution were similar between patients and healthy controls. In accordance, Al Rayes *et al.* and that the frequency of the G allele were similarly distributed between cases and controls [37]. Moreover, Zake *et al.* reported that both G allele and GG genotype were similarly distributed in cases and controls [43].

In contrast, Santos *et al.* [44] reported an association between A allele and RA. These studies showed that TNF-β +252 polymorphism together with HLA-DRB1*0405 has an influence on susceptibility to RA. Similarly, Al-Rayes *et al.* reported that GG genotype of TNF-β (+252) polymorphism was more frequent in RA patients as compared to the control group [37].

In the current study, analysis of the genetic influence of TNF-α 308 allele and on disease activity represented by DAS28 scoring system revealed that the G allele and GG genotype are more frequently represented in the non-remission group compared to the remission group ($p = 0.008$ and $p < 0.001$, respectively). This might be related to the influence of the SNPs, detected in genes coding for TNF-α and iNOS, on the severity of the inflammatory process, which might result in modification of RA disease activity [43].

In accordance with the results of the current study, Petra *et al.* and Hussein *et al.* indicated a positive association between GG genotype and disease activity [33, 45]. On the other hand, Nemec *et al.* did not detect an association between TNF-α 308 G/A promoter SNP and RA disease activity represented by disease activity score DAS28 scoring system [38].

In the current study, analysis of disease severity represented by Sharp score revealed that regarding TNF-α 308 G/A SNP Sharp score was highest among patients carrying the AG genotype compared to patients carrying the AA and GG genotypes ($p = 0.007$), the heterozygous mutant (AG). The effect of polymorphism on disease severity

could be explained by differences the rate of TNF-α synthesis. Thus, the production of TNF-α may be associated with TNF-α promoter SNP. In fact, the role of linkage disequilibrium is intense in this area, and it may be difficult to study the role of SNPs separately [38]. Moreover, circulating TNF-α levels might be under a complex regulatory process. Circulating TNF-α level is regulated at different stages: gene transcription, post transcription control of mRNA stability, cleavage of the membrane form to release the soluble form, and the expression of receptors [46].

Our results regarding radiological joint damage were in accordance with Rezaieyazdi *et al.* [47], who documented the association between heterozygous mutant (AG) genotype with a worse course of the disease. However, in contrast, Nemec *et al.* [38] reported an association between severe course of RA and TNF-α 308 GG genotype. This was also reported by Barton *et al.* [48], who reported that the G allele showed a tendency towards worse radiological outcome at five years, as measured by the presence or absence of erosions, in patients with inflammatory arthritis. On the other hand, Mosaad *et al.* reported that RA patients with A allele tend to have an increased number of erosions [38]. By contrast, there was no significant association between erosive disease and TNF-α, in Turkish patients and Polish patients [49].

In the current study, no statistically significant association was found between TNF-α 1031 T/C polymorphism and RA disease activity and severity. In agreement with the results of the current study, Barton *et al.* reported the absence of a significant association between TNF-α 1031 and disease severity in RA patients [48]. Contrary to our findings, Karray *et al.*, pointed out that the C allele and CC genotype were significantly higher in patients in remission of RA activity than in those from the non-remission group [27]. There was no statistically significant association detected as regards disease activity and severity and LTA 252 SNP. In agreement with our findings, Karray *et al.* and Al Rayes *et al.* also found no association with RA activity and severity [27, 37].

Conclusions

The results of the current study suggest that TNF-α 308 G/A SNP can be genetically associated with the susceptibility to RA in our study group and might be involved in both disease activity and severity. Therefore, the TNF molecule might have major genetic and/or functional involvement in the pathogenesis of RA and might also be implicated in disease activity and severity in the Egyptian patients.

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The authors declare no conflict of interest.

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