Int J Clin Exp Pathol 2016;9(10):9786-9797
www.ijcep.com /ISSN:1936-2625/IJCEP0034537

Original Article

Genetic variations of the TNF-α -308 G>A promoter, and TGF-β1 T869C polymorphisms in Egyptian patients with rheumatoid arthritis

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Received June 27, 2016; Accepted July 15, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Introduction: Rheumatoid arthritis (RA) is a complex autoimmuno disease. Tumor necrosis factor alpha (TNF-α), and transforming growth factor beta 1 (TGF-β1) are responsible for progress of RA. Aim: This study was to evaluate the prognostic implication of promoter polymorphism of TNF-α at 308 G/A, and T869C polymorphisms of TGF-β1 gene; in RA patients. Method: 42 RA patients; and 23 controls were included in this study. Physical and clinical features were performed. Serum TNF-α and TGF-β1 levels were enzymatically detected, in addition to TNF promoter (-308 G/A) genotyped was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); Moreover, single-nucleotide polymorphism T^{29}-C in exon 1 of the TGF-β1 gene was determined with RFLP. Results: Serum TNF-α was significantly increased in RA cases as compared to controls ($P<0.05$), while TGF-β1 level was not significant in RA cases ($P>0.05$). GG genotype was present in 52.4% of the RA patients, and 30.4% of controls ($P=0.005$). Susceptibility to RA was significant for G-allele (GG) TNF-α 308 ($P<0.001$; RR=1.26). While susceptibility to RA was not significant for TGF-β1 T869C polymorphism. Conclusion: The risk of developing RA was associated with TNF promoter (-308 G/A) genotypes.

Keywords: Rheumatoid arthritis, tumor necrosis factor, TNF-α -308 G/A polymorphism, T869C-TGF-β1 polymorphisms, TGF-β1

Introduction

Rheumatoid arthritis (RA) is a complicated autoimmune disease; it affects on about 1% of the world's population; It is an inflammatory disease which affect different organs; including; kidney, eyes, spleen, heart, and lungs [1]. RA characterized by; disturbance cytokine network, over expression of proinflammatory, and anti-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), and transforming growth factor beta1 (TGF-β1) which have been found in RA; TNF-α is an anti-inflammatory cytokine which acts as permanent cytokine in inflammatory, and autoimmunodisease, in addition to presence in synovial tissue of RA disease. TNF-α is a multifunctional cytokine in humans including enhancement of apoptosis, regulation of cell proliferation and having anti-inflammatory role in RA; lead to enhancement of apoptosis, regulation of cell proliferation. TNF-α gene is located within the highly polymorphic region on chromosome 6p21.3. High expression of TNF-α responsible for reactive oxygen species (ROS) release in RA patients, this lead to tissue damage associated with the inflammation [2].

There are different genetic variations in TNF-α gene such as single nucleotide polymorphisms (SNPs), these variation affect TNF-α protein expression. Polymorphism at -308 guanine (G) has been reported in different auto immune diseases as RA. The genetic variation at position -308 results in presence of two allelic features in which G is defines as the common variant; and the presence of adenine (A) defines as less common. Alleles A causes increase TNF gene expression to 6-7 folds as compared to G allele [3].

TGF-β1 is one of the TGF-β family proteins, which is secreted by variety types of cell with unique; and potent modulatory properties in the maintenance of normal immunological characterization. TGF-β1 gene is located in the 19q13 chromosome region. TGF-β1 displays many functions which are dependent on the cell type, and the state of differentiation [4].

TGF-β1 gene has several polymorphisms, including T869C, which have been shown to be associated with the serum level of TGF-β1. TGF-β1 is present at a high level in the synovial tissue, and play important role with combination of different cytokines in the progression of RA due to accumulation of neutrophil, in addition to it have immune-suppressive, and anti-inflammatory properties [5].

The aim of this study is evaluating implication prognostic value of TNF-α; and -308 G/A promoter polymorphism in RA patients; and its correlation with different demographic; and clinical parameters. Also; investigating the association between T869C polymorphisms of TGF-β1 gene; and development of RA.

Subjects and methods

Patients and controls

Forty-two of Rheumatoid arthritis patients with defined RA according to fulfillment of the American College of Rheumatology (ACR) criteria for the diagnosis, and for the classification of RA, were collected from the Rheumatology and Rehabilitation, and Internal Medicine departments, and their outpatient clinics of Cairo University hospitals; they were collected in the period from March 2012 till April 2013. Full history includes; age, sex, disease duration, clinical manifestation, and type of treatment. Disease activity score in 28 joints (DAS-28) were calculated according to DAS28= 0.56*√tender joints 28 (t28) + 0.28*√swollen joints 28 (sw28) + 0.70* Ln Erythrocyte sedimentation rate (ESR) + 0.014* vascular activity score (VAS) [6].

The Health Assessment Questionnaire-II (HAQ-II) was used [7]. In addition 23 healthy individuals with matched age, and sex were used as a control group. The study was approved by institute ethics committee, and the study conforms to the provisions of the Declaration of Helsinki in 1995. All patients gave their informed consent prior to their inclusion in the study.

Samples

3 mL venous blood was drawn with sterile syringe, and immediately transferred into a prelabeled blood collection vial containing anticoagulant (0.5 M EDTA). In addition to 2 mL of blood were allowed to clot at room temperature, and serum separated by centrifugation at 3000 r.p.m for 10 min, then serum was divided in to 2 tubes one of them for detection of serum TNF-α, and the other for detection of serum TGF-β1.

Quantitative detection of serum TNF-alpha, and TGF-β1 by ELISA method

TNF-alpha was assayed using a Human TNF-alpha ELISA kit (quantitative sandwich enzyme immunoassay technique), provided by Assaypro LLC. In addition to the serum concentration of TGF-β1 activation protein was determined with an enzyme-linked immunosorbent assay kit (RayBiotech, USA). Results were expressed as pg/mL.

Genomic DNA extraction

Genomic DNA was extracted from the peripheral blood of RA patients, and controls using QIAamp® DNA mini kit (Qiagen CA, USA). Enzymatic amplification was performed by a PCR using the Master TaqMan (Taq) polymerase enzyme, and thermal cycler (Applied Biosystems Gene Amp PCR System 9700, USA).

TNF-α gene (-308 G/A) promoter region polymorphism

Promoter region polymorphism of TNF-alpha gene (-308 G/A) was determined by PCR-RFLP technique. Amplification of the promoter region (-308 G/A) of the TNF-alpha gene was performed as proposed by Dalziel et al [8] using 2 primers which were purchased from Operon Biotechnologies (Sigma, USA). The primers are used to amplify target DNA in the promoter region (-308 G/A) of the TNF-alpha were summarized in **Table 1**.

The PCR reaction mixture (25 μL) contained 12.5 μL 2 × PCR Master Mix (10 × PCR buffer), 4 mM $MgCl_2$, 0.5 Taq DNA polymerase/μL, and

Table 1. Primers used to amplify DNA containing position -308 of the TNF-α, and T869C polymorphisms of the TGF-β1 gene

Primers	Sequence	Fragment band	Digest Enzyme
Sense	5'-AGGCAATAGGTTTTGAGGGCCAT-3'	107 bp	NcoI
Anti-sense	5'-TCCTCCCTGCTCCGATTCCG-3'		
Sense	5'-TCCGTGGGATACTGAGACACC-3'	241 bp	PvuII
Anti-sense	For First Allele 5'-GCAGCGGTAGCAGCAGCG-3' For Second Allele 5'-AGCAGCGGTAGCAGCAGCA-3'		

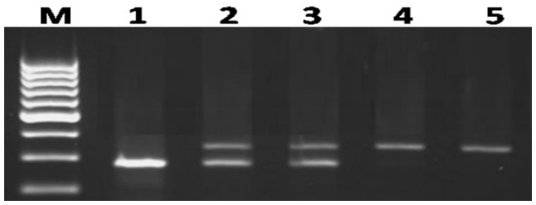

Figure 1. Agarose gel electrophoresis of TNF-α product after digestion with NcoI restriction enzyme. M lane: DNA Ladder (50 bp). Lanes 1: homozygous GG genotype yielded 1 band of 87 bp. Lanes 2 and 3: heterozygous GA genotype yielded 2 bands of 107 bp and 87 bp. Lanes 4 and 5: homozygous AA genotype yielded 1 band of 107 bp.

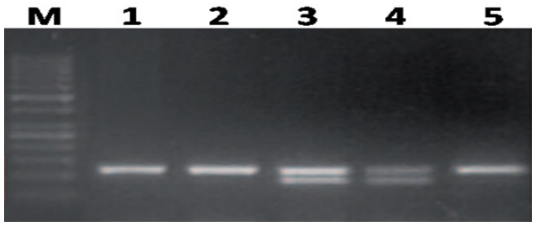

Figure 2. Agarose gel electrophoresis of TGF-β1 products after digestion with PvuII restriction enzyme. M lane: DNA Ladder (50 bp). Lanes 1, 2 and 5: homozygous CC genotype yielded 1 band of 153 bp. Lanes 3 and 4: heterozygous TC genotype yielded 2 bands of 153 bp and 135 bp.

0.4 mM dNTPs (dATP, dCTP, dGTP, dTTP), 1 μL of each primer (25 pmol), 3 μL of genomic DNA, and 7.5 μL sterilized nuclease-free water. The reaction was carried out with the following cycles: 1 cycle of 95°C for 2 min; 35 cycles of 95°C for 40 s, 60°C for 40 s, 74°C for 40 s, and 1 cycle of 74°C for 5 min for final extension. Then amplified products were digested with 5 units Fast Digest NcoI restriction enzyme at 37°C for 10 min supplied by (New England Bio labs). The resultant products were analyzed by electrophoresis in 2.5% agarose gel, and stained with ethidium bromide, and visualized by UV light (Promega, USA) for the identification of TNF-alpha gene (-308 G/A) promoter polymorphism.

A single band at 107 bp identified AA homozygous individuals, one bands at 87 bp identified GG homozygous individuals, and two bands at 107, and 87 bp indicated a heterozygote at the TNF-α -308 locus [9] (**Figure 1**).

T869C polymorphisms of the TGF-β1 gene

T869C polymorphism of the TGF-β1 gene was determined using PCR-RFLP as previously described. The PCR primers that were used were shown in **Table 1**.

Sample DNA (100 ng) was amplified in 25 μL of a reaction mixture containing 0.5 units of Taq DNA polymerase, dNTPs (2.5 μM each) (Perkin-Elmer, Norwalk, CT, USA), using an automated PCR thermal cycler (GeneAmp PCR System 2700; Applied Biosystems; Foster City, CA USA). The samples were denatured at The thermo cycling procedure was as follows: initial denaturation at 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The PCR products were digested for 2 h at 37°C with PvuII (10,000 units/mL) (New England Biolabs, Hitchin, U.K), and 10 μL amplicon, run on a 1.5% agarose gel, and then visualized with ethidium bromide. TT genotype yields only a single 135 bp band, while the CT result in two band 153 bp, and 135 bp, and CC genotype yield single band 153 bp (**Figure 2**). The PCR-RFLP results were confirmed in selected cases by direct genotyping (ABI PRISM 310 Genetic Analyzer; Applied Biosystems; Foster City, CA USA).

Statistical analysis

Data were collected, tabulated, and analyzed by SPSS package version 20 (SPSS corporation, USA). Data were summarized as mean ± SD. Genotypes and allele frequencies were represented as percentages, and the frequencies were calculated by gene counting method. Genotypic distributions in the patients and healthy control subjects were analyzed with

Table 2. Demographic, clinical and laboratory features of the Egyptian rheumatoid arthritis patients

Characteristic	RA (n=42)	Control (n=23)
Sex		
Female n (%)	36 (85.7)	21 (91.3)
Male n (%)	6 (14.3)	2 (8.7)
F:M ratio	6:1	10.5:1
Age		
Mean ± SD (years)	43.47±12.73	31.70±7.87
Range	26-70	23-65
Disease Duration (month)	65.62±49.24	-
Morning stiffness		
Mean ± SD (minutes)	26.12±36.3	-
Range	0-120	-
Swollen joints count		
Mean ± SD	2.81±5.4	-
Range	0-18	-
Tender joint count		
Mean ± SD	4.33±5.7	-
Range	0-20	-
RA activity n (%)		
High DAS28>5.1	6 (14.3)	-
Moderate 3.2<DAS28<5.1	12 (28.6)	-
Low DAS28<3.2	16 (38.1)	-
Remission DAS28<2.6	8 (19)	-
ESR (mm/1st h)		
Mean ± SD	34.24±37.1	ND
Range	5-125	
Hemoglobin (g/dL)		
Mean ± SD	11.63±1.7	ND
Range	9-14	
Platelets		
Mean ± SD	234.9±49.4	ND
Range	134-369	
Metabolic bone n (%)	2 (3.7)	-
Osteoporosis n (%)	6 (11.1)	-
SCN n (%)	4 (7.4)	-

Data were expressed by mean ± SD and range, in addition, number and percent.

Table 3. Serum TNF-α, and TGF-β1 in studied groups

Parameter	RA (n=42)	Control (n=23)	P
TNF-α (pg/mL)	35.1±14.64	8.98±6.69	<0.001***
TGF-β1 (pg/mL)	2349.05±647.6	2456.5±649.9	0.476

Data were expressed as mean ± SD; NS, $P \geq 0.05$; ***$P \leq 0.001$.

Fisher's exact test. The strength of the association of disease with respect to a particular allele/genotype was expressed by odd ratio interpreted as *relative risk* (RR) following the Woolf's method. It was calculated only for those alleles/genotypes which were increased or decreased in arthritis patients as compared to control group. The RR was calculated for all the subjects using the formula given below:

$RR = (a) \times (d)/(b) \times (c)$

a = number of patients with expression of allele or genotype; b = number of patients without expression of allele or genotype; c = number of controls with expression of allele or genotype; d = number of controls without expression of allele or genotype.

Spearman's correlation analysis was used for the detection of the relation between 2 variables. Logistic regression analysis was applied to detect the predictors for the elevated TNF-α level, TGF-β1, and their polymorphism. Results were considered significant at $P<0.05$.

Results

Patient's characteristics

In this study, there were 42 RA patients, mean age of rheumatoid arthritis patients their ages ranged from 26 to 70 years was 43.48±12.73 years, in addition 23 healthy individuals with matched age, and sex were used as a control group, their ages ranged from 23 to 65 years with a mean 31.70±7.87. The demographic and clinical characteristics of the RA patients and controls are summarized in **Table 2**.

Prognostic value of serum TNF-α, and TGF-β1 in rheumatoid arthritis patients, and control

Table 3; **Figure 3**, show there was highly significant increase in serum TNF-α in RA group as compared with control group ($P<0.001$). There were none statistically significant negative correlation between TNF-α, and age (r=-0.156, P=0.324), ESR (r=-0.097, P=0.542), DAS28 (r=-0.208, P=0.187), and platelets count (r=-0.323, P=0.053). Also, there was none statistically significant decrease in serum TGF-β1 in rheumatoid arthritis group as compared with

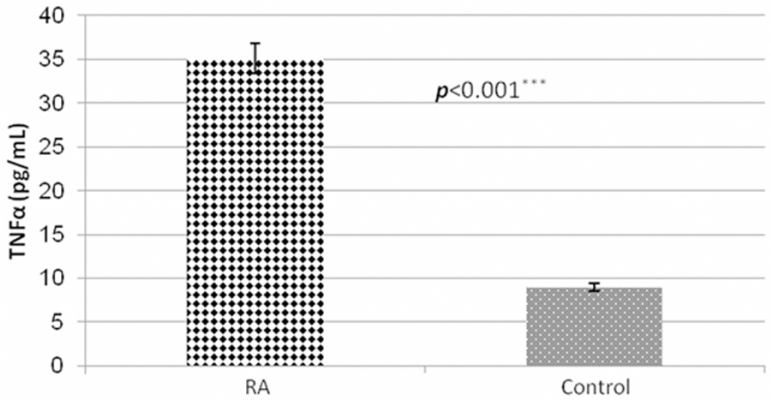

Figure 3. Serum TNF-α in studied group.

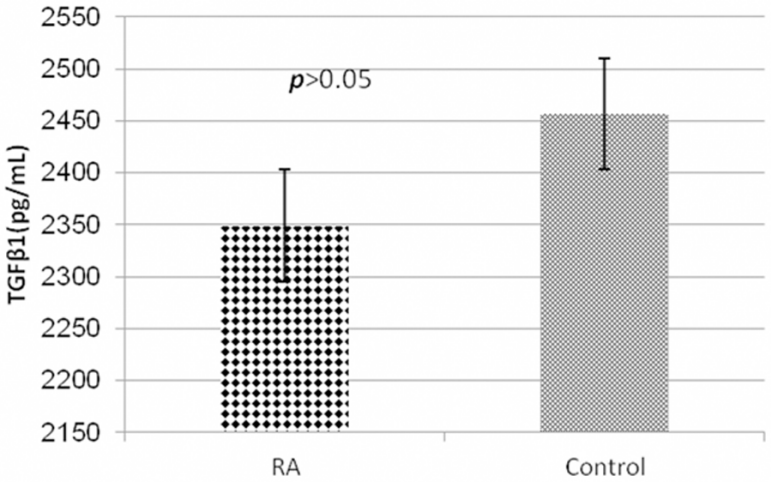

Figure 4. Serum TGF-β1 in studied group.

Table 4. Genotype and allele frequencies of TNF-α (G-308A) polymorphism in RA patients and controls

Genotype/ Allele	RA (n=42)	Control (n=23)	*P*-value	RR	OR	95% CI
	n (%)	n (%)				
GG	22 (52.4%)	7 (30.4%)	0.005**	1. 7	2.51	0.8581-7.3667
GA	16 (38.1%)	12 (52.2%)	0.45	0.73	0.56	0.2017-1.5773
AA	4 (9.5%)	4 (17.4%)	0.362	0.55	0.5	0.1125-2.2215
GA and AA	20 (47.6%)	16 (69.6%)	0.093	0.68	0.398	0.1357-1.1653
G-allele	60 (71.4%)	26 (56.5%)	<0.001***	1.2	1.923	0.908-4.075
A-allele	24 (28.6%)	20 (43.5%)	0.088	0.67	0.5	0.2454-1.1019

Data were expressed as number and percent; **$P \leq 0.01$; ***$P \leq 0.001$.

control group (P>0.05). There were none statistically significant negative correlation between TGF-β1, and age (r=-0.124, P=0.432), ESR (r=-0.063, P=0.691), DAS28 (r=-0.085, P=0.592), on the other hand there was a significant positive correlation between TGF-β1, and platelets count (r=0.474, P=0.002**) also, there was a significant negative correlation between TGF-β1, and TNF-α (r=-0.338, P=0.028*) **Figure 4**.

Genotypes and alleles frequencies of TNF-α 308 promoter polymorphism in studied groups

The genotypes and alleles frequencies of TNF-α -308 promoter polymorphism in RA patients, and control individuals were presented in **Table 4**; **Figure 5**. The frequency of allele-A was none significantly lower in RA patients as compared to control group (*P*=0.546, RR=0.66), on the other hand the frequency of allele-G was higher in RA patients as compared to the controls (P<0.001, RR=1.26). The homozygous GG genotype was present in 52.4% of the RA patients, and 30.4% of controls (P= 0.005), while heterozygous GA was found in 38.1% of RA patients against 52.2% of controls (P=0.45). The homozygous AA genotype was found in 9.5% of RA patients, and 17.4% of control samples (P=1.0). Allele-A containing genotypes (GA and AA) were present in 47.6% of patients, and 69.6% of the healthy controls (P=0.505, RR=0.68). Susceptibility to RA was significant for homozygote for TNF-α 308 G-allele (GG) (P< 0.001; RR=1.26).

T869C polymorphisms of the TGF-β1 gene in studied groups

From 42 patients with RA, 10 (23.8%) were TT type, 24 (57.1%) were CT, and 8 (19%) were CC type as shown in **Table 5**; **Figure 6**, the difference in genotypic distribution between the controls, and patients was not statistically significant when we compared all three genotypes.

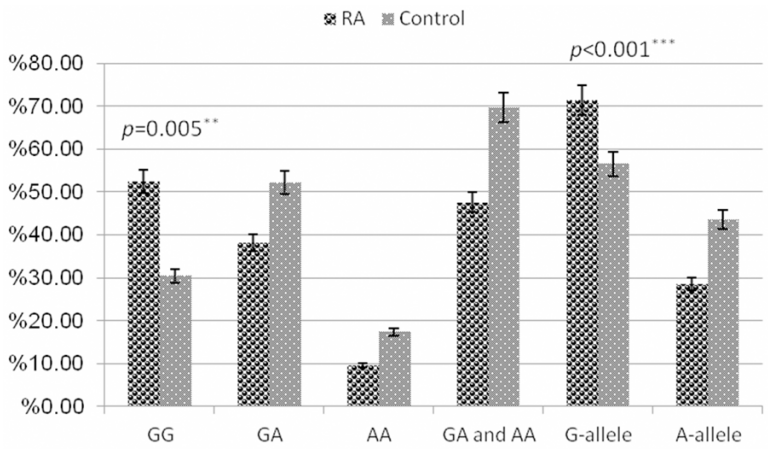

Figure 5. Genotype and allele frequencies of TNF-α (G-308A) polymorphism in RA patients and controls.

Table 5. Genotype and allele frequencies of transforming growth factor-β1 T869C polymorphism in RA patients and controls

Genotype/ Allele	RA (n=42)	Control (n=23)	*P*-value	RR	OR	95% CI
	n (%)	n (%)				
TT	10 (23.8%)	5 (21.7%)	0.849	1.1	1.13	0.3325-3.8068
TC	24 (57.1%)	10 (43.5%)	0.29	1.3	1.7	0.6211-4.8369
CC	8 (19%)	8 (34.8%)	0.146	0.55	0.44	0.1393-1.3976
TC and CC	32 (76.2%)	18 (78.3%)	0.85	0.97	0.89	0.2627-3.0078
T-allele	44 (52.4%)	20 (43.5%)	0.65	1.2	1.4	0.6938-2.9476
C-allele	40 (47.6%)	26 (56.5%)	0.332	0.84	0.67	0.3393-1.4414

Data were expressed as number and percent; NS, *P*>0.05.

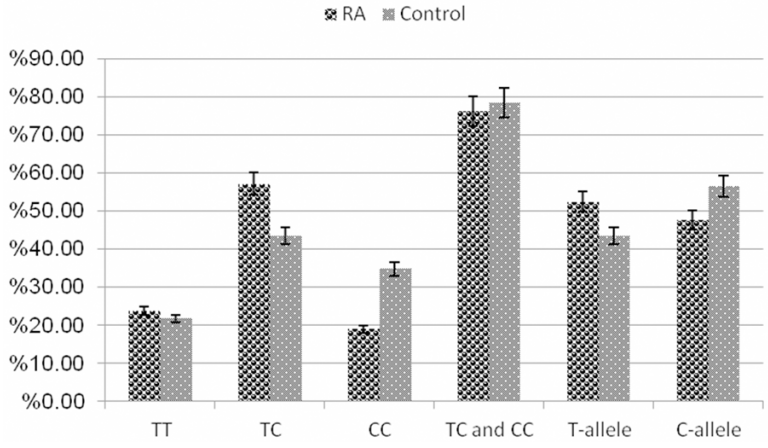

Figure 6. Genotype and allele frequencies of transforming growth factor-β1 T869C polymorphism in RA patients and controls.

Association between demographical and clinical characteristics, and TNF-α (308 G/A) genotypes

The relation between gene polymorphism, clinical, and laboratory data patients was showed in **Table 6**. Patients with AG genotype show significant increase in the count of tender joint or the level of DAS 28 as compared to the GG or AA genotype. On the contrary ANOVA test showed none significant between the counts of tender joint with different genotype. As well as, the result showed that there was significant increment between the patients with AA in the level of platelets when compared to the GG or AG genotypes. The TNF-α was significantly higher in patients with GG (40.05±13.96 pg/mL) compared to those with GA (34.25±11.19 pg/mL), and AA (11.3±0.23 pg/mL), (P< 0.001) (**Figure 7**).

Furthermore, there was none significant difference in morning stiffness (minutes), swollen joints count, tender joints count, and platelets according to the three gene promoters (P=0.451, 0.461, 0.602, 0.331, 0.306 and 0.436 respectively). But, there was significant difference in ages, DAS28, and ESR according to the three gene promoters (P<0.001, 0.007, and 0.011 respectively).

TGF-β1 genotypes and clinical characteristics of patients with RA

The relation between genotypes and clinical characterization was showed in **Table 7**. Patients with CC genotype show significant increase in the level of DAS 28, and ESR level as compared to the TT or CT genotype. On the other hand, platelets count was statistically significant increase in CT genotype as compared to the TT or CT genotype. On the contrary ANOVA test showed none significant between the counts of tender joint with different genotype. TGF-β1 levels were none statistically significant

Table 6. The association between demographical and clinical characteristics and TNF-α (308 G/A) genotypes in patients with rheumatoid arthritis

	GG (n=22)	AG (n=16)	AA (n=4)	*P value*
Sex (M/F)	2/20	4/12	0/4	0.266
Age (years)	37.36±7.96	51.12±14.94	46.50±7.50	0.002*
Disease Duration	20.27±33.72	60.75±58.48	42.0±20.78	0.453
morning stiffness	20.27±33.71	37.50±41.87	12.50±8.66	0.264
swollen joints count	2.18±4.32	4.25±7.09	0.50±0.57	0.351
tender joints count	3.18±5.70	6.87±5.66	0.50±0.57	0.052
DAS28	3.03±1.28	4.60±1.69	3.55±0.24	0.007**
ESR (mm/1st h)	18.45±23.68	51.75±46.18	51.0±18.47	0.012*
Hemoglobin (g/dL)	11.56±1.26	11.42±2.12	12.80±1.62	0.337
Platelets	226.54±42.83	229.37±39.67	303.0±76.21	0.011*
TNF-α (pg/mL)	40.05±13.96	34.25±11.19	11.30±0.23	<0.001***

Data were expressed as mean ± SD; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; NS, $P > 0.05$.

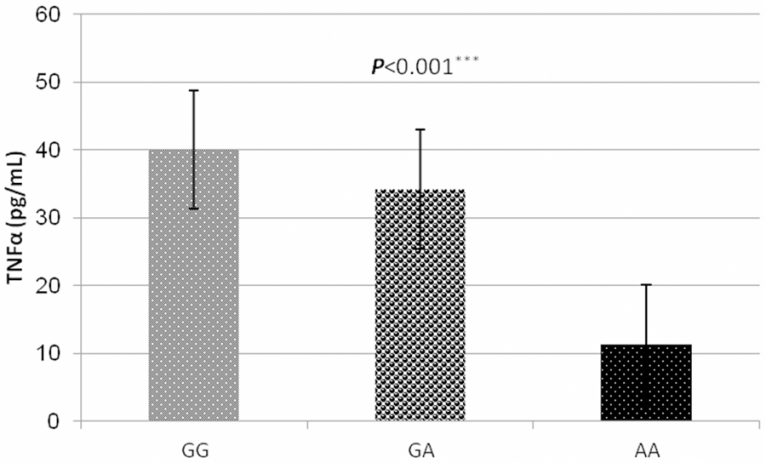

Figure 7. Serum TNF-α levels in the RA patients according to the TNF-α (-308 G/A) promoter polymorphism type (GG, GA, and AA genotypes).

higher in patients with CC (2444±1088.89 pg/mL) compared to those with CT (2410±277.3 pg/mL), and TT (2289.17±501.24 pg/mL), (P=0.869) (**Figure 8**).

Furthermore, there was non-significant difference in age at disease onset, disease duration, morning stiffness (minutes), swollen joints count, tender joints count, hemoglobin, and TGF-β1 according to the three gene promoters (P=0.125, 0.943, 0.909, 0.877, 0.609, 0.725, and 0.869 respectively). But, there was significant difference in DAS28, ESR, and platelets according to the three gene promoters (P=0.045, <0.001, and 0.042 respectively).

Ethnic variations in genotype distribution of TNF-α (-308) promoter

The comparison between the frequencies of genotypes of TNF-α (G-308A) promoter polymorphism in RA Egyptian population with the frequencies reported for RA population of various ethnicities worldwide was presented in **Table 8**.

These results clearly indicated ethnic variations in genotype distribution of TNF-α (-308) promoter polymorphism.

Genotype distribution of TGF-β1 according to ethnic variations

Table 9, show there was different in genotype distribution of TGF-β1 according to ethnic variations.

Discussion

RA is a complex auto immune disease. Unbalanced cytokine is responsible for the pathogenesis of different infections; and inflammatory diseases. Any defect in gene expression that increases the cytokine production; may be altering the homeostasis of the organism; lead to organ systemic function failures [23]. TNF is one of these cytokines which has been described to play provital role in these processes. TNF-α plays a major role in pathogenesis of different auto-immune diseases such as RA [24].

TNF-α plays a key function in the initial, and prolonged inflammation; and in joint destruction, controlling the production of interleukin 1 (IL-1); and other proinflammatory cytokines including interleukin-6 (IL-6); and interleukin-8 (IL-8). Any change in TNF-α responsible for over-

Table 7. The association between demographical and clinical characteristics and TGF-β1 (308 G/A) genotypes in patients with rheumatoid arthritis

	TT (n=10)	TC (n=24)	CC (n=8)	*P value*
Sex (M/F)	0/10	4/20	2/6	0.129
Age (years)	51.75±18	41.8±9.88	41.42±11.08	0.125
Disease Duration	76.22±45.5	57.67±51.35	65.62±49.2	0.943
Morning stiffness	8.45±8	120±0.01	37.5±33.3	0.909
Swollen joints count	1±1.3	15.67±1.86	0.73±0.99	0.877
Tender joints count	6±7.37	3.08±3.88	4.33±5.75	0.609
DAS28	3.68±1.39	3.13±1.3	5.33±1.46	0.045*
ESR (mm/1st h)	18.58±20.93	24.2±22.8	93.75±30.09	<0.001***
Hemoglobin (g/dL)	12.34±1.68	10.9±0.66	11.63±1.68	0.725
Platelets	232.25±33.1	259.2±67.8	212.5±57.95	0.042*
TGF-β1 (pg/mL)	2289.17±501.2	2410±277.3	2444±1088.89	0.869

Data were expressed as mean ± SD; *$P \leq 0.05$; ***$P \leq 0.001$; NS, $P > 0.05$.

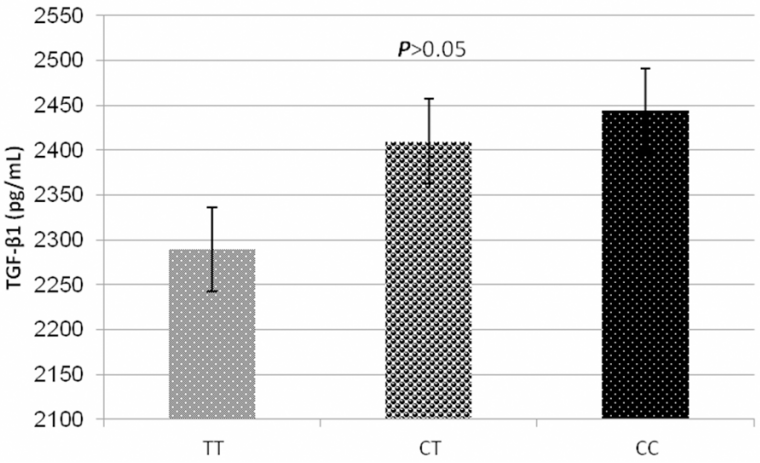

Figure 8. Serum TGF-β1 levels in the RA patients according to the TGF-β1 T869C Polymorphism type (TT, CT, and CC genotypes).

Table 8. Genotype distribution of TNF-α (-308) promoter Polymorphism in different RA groups

Population/group studied	Genotypes		
	GG	GA	AA
Egypt [1]	52.40	38.1	9.5
Saudis [10]	50.0	38.09	11.90
American [11]	68.2	29.1	2.7
Australian [12]	58.0	39.0	3.0
Chilean [13]	83.1	16.3	0.6
Chinese [14]	83.2	15.7	1.1
Danish [15]	65.8	30.5	3.7
Dutch [16]	56.6	38.6	4.5

production of TNF-α; worse outcome; and disease severity. More of polymorphisms have been studied in the promoter region of TNF-α gene. G>A substitution at -308 position polymorphism was one of the most extensively investigated variant. Many studies have suggested that there was association between 308 G>A polymorphism; and higher levels of TNF-α transcription [25]. Elevated TNF levels due to the -308 polymorphism may alter the immune response; increase rate of infectious; and autoimmune diseases. It has been suggested that severe genetic factors are involved in the pathogenesis of RA. In this study, we have investigated the association of RA with TNF-α; and G/A -308 position polymorphism.

TGF-β1 is one of important cytokines; which negatively effect on immune response. TGF-β1 is the major member of a family which have role in cell growth; and differentiation. TGF-β1 is produced from different type of immune system cell as macrophage, T lymphocyte; and B lymphocyte. There was antigonistics correlation between TNF-α1; and TGF-β1. TGF-β1 inhibits the production of TNF-α1 by cells in RA; this may be due to its immunosuppressive effect on cytokine production.

One of the main functions of TGF-β1 in immune system suppresses the lymphocyte development; proliferation; and this effect on the suitable immune response. We were studied association between TGF-β1 T869C polymorphisms; and TGF-β1 protein concentrations [26].

In this study, in RA group females were more affected than males (85.7%). This finding was in line with other studies from populations of France, America, Taiwan, and Netherlands which have also reported higher percentage of female RA patients [27].

Table 9. It shows there was different in genotype distribution of TGF-β1 according to ethnic variations

Population/group studied	Genotypes		
	TT	TC	CC
Egypt [17]	52.40	38.1	9.5
Japan [18]	21.9	59.4	18.70
New Zealand [19]	27.4	49.6	23.1
China [20]	31.6	53.9	14.5
Turkey [21]	42.7	41.2	16
UK [22]	40.8	44.6	14.7

This result indicated that there was association between sex; and RA disease, RA increase in female than in male; this may be due to distribution of sex hormone; where estrogen produced in female; and this enhancement antibody production; which suppressed by androgen produced in male [28].

In this study there was highly significant increase in serum TNF-α in RA patients as compared with control group (P<0.001), this associated with the increased polymorphisms in its gene promoter region.

This conformed to presence of high TNF-α level in the synovial fluids; and tissue of RA patients [25]. This agree with Tamer study's [1], who decided that there was elevation in TNF-α levels in RA patients as compared with control group (P=0.036).

Elevated TNF levels due to the -308 polymorphism may be result in alter the immune response, and this lead to infectious; and autoimmune diseases as RA.

Hussein et al [29] reported that there was a significant association of TNF-α -308 alleles with RA.

On the other hand; there was none statistically significant decrease in serum TGF-β1 in RA patients as compared with healthy control. This decrease may be due to migration of lymphocytes to epitheliums or due to genetic abnormalities. There was another reason for reduction of TGF-β1 immune injury in salivary gland increase the rate of consume of TGF-β1 in process of repair and fibrosis mechanism. The lowest levels of TGF-β1 in serum of patients with RA might reflect immune injury; spreading; and severity of autoimmunity [30].

In previous studies the T allele of T869C polymorphism has been reported to be associated with reduced of TGF-β1 protein level.

In this study, there was significant lower frequency of -308A allele and higher frequency of -308G allele in RA patients as compared to healthy controls this result similar to the findings reported in various other ethnic groups including Saudis, American, Australian Chilean Chinese Danish, and Dutch [10].

G allele may have a protective role from the development of RA in Egyptians [1]. In the light of these results we reviewed the literature of available studies to clarify the role of -308 G/A polymorphism of the TNF gene in development of RA. There is positive association between RA disease and genetic.

In this study, the frequencies of CT, and TT T869C polymorphisms genotype of TGF-β1 in RA patients were higher than those in control subjects (P>0.05), the frequencies of T allele (52.4%) were higher, and C allele (47.6%) were lower in RA group than those in the controls (P>0.05).

C allele may have a protective role from the development of RA in Egyptians RA patients [17].

Zhou et al, [31] found that T869C TT genotype in the overall population was associated with increased RA risk (OR=1.28, 95% CI: 1.02-1.60, P=0.03).

In a meta-analysis study, TNF-α -308 A/G polymorphism represented a significant risk factor for RA in Latin Americans; but not in Europeans which favors that different ethnic populations influence this association [32].

The -308 G/A polymorphism was significantly associated with a poor outcome in the Turkish juvenile idiopathic arthritis (JIA) patients (P= 0.005); but there was no association in the Czech patients.

In this study, TNF-α -308 AA allele showed increased frequency in severe RA when compared to non-severe disease and to healthy controls. In a meta-analysis study, T allele TGF-β1 polymorphism represented a significant risk factor for RA. Difference in the frequency of genotype, and allele in different ethnic popula-

tions or studies might due to genetic abnormalities, sex, age; and disease severity all of this responsible for change in results [33].

In this study, there was not significantly difference in morning stiffness (minutes), swollen joints count, tender joints count; and platelets according to the three TNF-α gene promoters, on the other hand, there was significant difference age, DAS28; and ESR according to the three TNF-α gene promoters in RA patients.

This in coincided to [1] who showed that there were negative correlation between age, DAS28; and ESR regarded to TNF promoter polymorphisms at position -308.

In the current study, the serum TNF-α level of the G/A patients were lower than those of the G/G patients. Disease activity enhanced G/G genotype as compared with GA or AA. In JIA patients with the genotype -308 GG achieved an obvious improvement in disease activity than those with the genotype -308 GA or AA.

Patients not responding to anti-TNF-α therapy characterized with an increased frequency of the A allele. The significant association between the TNF-α promoter -308 A/G polymorphism; and responsiveness to anti-TNF therapy, suggests that RA patients who carry the A allele have worse response to therapy than those with the G allele, on the other hand patients with a TNF-α -308 G/G genotype were better responders to anti-TNF-α treatment than those with A/A or A/G genotypes [34].

Also, there was not significantly difference in age, morning stiffness (minutes), swollen joints count, tender joints count, and hemoglobin according to the TGF-β1 genotypes. On the other hand, there was significant difference DAS28, ESR, TNF-α, and platelets according to the TGF-β1 genotypes in RA patients. Statistically positive correlation between TGF-β1, and platelets might due to most TGF in serum appears to be derived from platelets, which contain two pools of latent TGF1. One pool, containing the latent TGF-β binding protein and the mature TGF-β1 dimer, is released into the serum during clotting process. Statistically negative correlation between TGF-β1; and TNF-α might due to increase TGF-β1 inhibit the secretion of anti-inflammatory cytokines as TNF-α; and lead to prognosis of RA [35].

In the current study, the serum TGF-β1 levels of the CC patients were higher than those of the CT, and TT patients.

T alleles of T869C polymorphism might be associated with relatively low production of TGF-β1, and may correlate with the rate of progression of the disease; reduced TGF-β1 may result in increased inflammation in RA as result of disturbance in signaling peptide; and this effect on the pathway of different metabolic process.

T869C TT genotype was shown to be a risk factor for RA and T869C C allele or CC genotype shown to be a protective factor against RA disease [36].

In conclusion, these results indicated that TNF-*α* was inflammatory cytokines in RA; and genetic mutation as *TNF-α* -308 polymorphism; lead to development of RA. G allele at *TNF-α* -308 was more the common in RA patients; and controls. A allele was relatively less common in these subjects. T869C polymorphism of the TGF-β1 gene was not associated with development of RA. The results of this study may have prognostic value for future clinical observations of RA patients; and investigate the sensitivity of patients to treatment.

Disclosure of conflict of interest

None.

Abbreviations

RA, rheumatoid arthritis; TNF-α, tumor necrosis factor alpha; TGF-β1, transforming growth factor beta 1; ROS, reactive oxygen species; SNPs, single nucleotide polymorphisms; G, guanine; A, adenine; ACR, American College of Rheumatology; SCN, severe congenital neutropenia; ESR, erythrocyte sedimentation rate; DAS28, disease activity score 28; OD, odd ratio; RR, relative ratio; VAS, vascular activity score; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; t28, tender joints 28; sw28, swollen joint 28; Taq, TaqMan; RR, relative risk.

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