



Contents lists available at ScienceDirect

## The Egyptian Rheumatologist

journal homepage: [www.elsevier.com/locate/ejr](http://www.elsevier.com/locate/ejr)

# Association of tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) –308A/G (rs1800629) gene polymorphism with carotid artery atherosclerosis in rheumatoid arthritis patients

Rasha M. Fawzy<sup>a,\*</sup>, Gamal A. Hammad<sup>a</sup>, Samy E. Egila<sup>a</sup>, Amira N. Elkasas<sup>a,b</sup>, Nehad A. Fouad<sup>c</sup>

<sup>a</sup> Department of Rheumatology, Rehabilitation and Physical Medicine, Faculty of Medicine, Benha University, Egypt

<sup>b</sup> Om El Masreen Hospital, Ministry of Health, Benha, Egypt

<sup>c</sup> Medical Microbiology and Immunology, Benha University, Egypt

## ARTICLE INFO

### Article history:

Received 18 April 2020

Accepted 17 May 2020

Available online 28 May 2020

### Keywords:

Atherosclerosis

Rheumatoid arthritis

Tumor necrosis factor (TNF- $\alpha$ )

## ABSTRACT

**Background:** Increased coronary artery atherosclerosis in rheumatoid arthritis (RA) may cause significant mortality. Tumor necrosis factor (TNF) is a potent proinflammatory cytokine that has been involved in RA pathogenesis and atherosclerosis.

**Aim of the work:** To determine the association between TNF- $\alpha$  rs1800629 polymorphism and carotid atherosclerosis in RA patients.

**Patients and methods:** This study was carried out on 50 RA patients and 40 age and sex matched healthy control. All patients were subjected to full history taking, thorough clinical examination, and assessment of disease activity score (DAS28). Carotid artery intima-media thickness (IMT) was measured by Doppler ultrasonography. TNF- $\alpha$  –308A/G (rs1800629) polymorphism was assessed.

**Results:** The mean age of patients was  $41.2 \pm 13.2$  years with disease duration of  $6.1 \pm 4.5$  years. The mean DAS28 was  $4.1 \pm 0.8$  and Larsen score  $2.1 \pm 0.9$ . The mean IMT was significantly higher in patients ( $0.78 \pm 0.47$  mm) compared to the control ( $0.44 \pm 0.16$  mm) ( $p < 0.001$ ). Carotid plaques and calcifications were present in 2 and 3 patients respectively. Regarding the TNF- $\alpha$  polymorphism, there was a significantly higher frequency of GG (60%) followed by GA (24%) and AA (16%) ( $p < 0.0001$ ). All control had GG genotype except 1 patient had GA. The G allele was significantly increased (72%) compared to the A allele (28%) ( $p < 0.0001$ ). The mean carotid IMT was significantly higher in AA genotype ( $1.2 \pm 0.4$  mm) compared to GA ( $1.02 \pm 0.5$  mm) and GG ( $0.5 \pm 0.3$  mm) ( $p < 0.001$ ).

**Conclusion:** Rheumatoid arthritis and atherosclerosis are strictly linked to each other; TNF- $\alpha$  –308A/G polymorphism might increase atherosclerotic susceptibility in RA patients through increased risk of inflammation with subsequent abnormal lipid profile.

© 2020 Egyptian Society of Rheumatic Diseases. Publishing services provided by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Rheumatoid arthritis (RA) is a worldwide inflammatory arthritis [1] that primarily targets the synovial membrane leading to synovial inflammation and proliferation, loss of articular cartilage, and erosions of juxta-articular bone causing progressive joint damage and deformity [2]. Interestingly, the clinical significance of atherosclerosis and carotid intima-media thickness (CIMT) has been investigated in Egyptian patients with various rheumatic diseases including systemic lupus erythematosus [3], psoriatic arthri-

tis [4], systemic sclerosis [5], Behcet's disease [6] and RA [7]. RA patients are more liable for developing coronary artery disease with a 1.5–2.0-fold increased risk compared with the general population [8] with increased mortality rate [9]. Chronic inflammation and drug-related risk factors have been suggested to cause a key role in the development of early atherosclerosis, which contributes to the development of cardiovascular disease (CVD) [10].

Tumor necrosis factor (TNF- $\alpha$ ) is one of the most important cytokines that causes inflammation in RA [9]. Monocytes and macrophages are the main producers of TNF- $\alpha$ , but also by B-cells, T-cells, and fibroblasts. Blocking TNF- $\alpha$  with antibodies significantly decreased the production of interleukin (IL)-1, IL-6, IL-8, and GM-CSF [10]. It has been established that TNF- $\alpha$  has numerous biological activities. As a starting point for endothelial dysfunction and endometrial thickening, it can damage directly the vascular

Peer review under responsibility of Egyptian Society of Rheumatic Diseases.

\* Corresponding author at: Faculty of Medicine, Benha University, Kalyoubia, Egypt.

E-mail address: [dr.rasha.fawzy@hotmail.com](mailto:dr.rasha.fawzy@hotmail.com) (R.M. Fawzy).

<https://doi.org/10.1016/j.ejr.2020.05.004>

1110-1164/© 2020 Egyptian Society of Rheumatic Diseases. Publishing services provided by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

endothelial cells with subsequent increase permeability resulting in more cholesterol deposited in the vascular wall, forming atherosclerotic plaques [11]. Besides it also potentiates the formation of platelet-derived growth factor, breaking balance between blood coagulation–anticoagulant, and contributing to thrombosis [12]. Additionally TNF- $\alpha$  diminishes lipoprotein activity, and affects the synthesis of other inflammatory factors [11].

It was hypothesized that RA is favored by the existence of the –308 TNF promoter polymorphism which augments the production of TNF [13]. It is located on chromosome six within the major histocompatibility complex. Furthermore, it is substantiated that there is a great link between TNF- $\alpha$  and/or its gene –308 G/A promoter polymorphism with SLE [14] and JRA [15] pathogenesis and severity.

This study designed to determine the association of TNF- $\alpha$  rs1800629 polymorphism with carotid atherosclerosis observed in RA patients.

## 2. Patients and methods

This case-control study was conducted on fifty RA patients who fulfilled the 2010 American College of Rheumatology classification criteria for RA [16] were recruited from the outpatient clinic and inpatient unit of Rheumatology, Rehabilitation and Physical Medicine Department, the practical part of the study was done at Medical Microbiology and Immunology Department of Benha University Hospitals. Forty healthy volunteers were recruited from the hospital personnel and matched for age and sex to RA patients as a control group. Any patient or control with a history of a cardiovascular event; ischemic heart disease (IHD), heart failure, cerebrovascular accident, or peripheral arteriopathy and smokers were excluded. The study was approved by the local university ethics committee. All patients gave their informed consent prior to their inclusion in the study.

Patients were subjected to full history taking, thorough clinical examination, and disease activity score in 28 joints was calculated [17]. Laboratory investigations included complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) and lipid profile: total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglyceride.

Carotid ultrasonography was performed to assess the carotid IMT and any areas of focal plaques with a GE Vivid 7 system (GE Healthcare, Milwaukee, WI) equipped with a 13 MHz linear array imaging probe. The right common carotid artery was scanned with the patient lying supine with his/her neck slightly extended and head directed away from the side of examination. A region 1 cm proximal to the carotid bifurcation was identified, and the intima media thickness of the far wall was evaluated as the distance between the lumen–intima interface and the media–adventitia

interface. The IMT measurement was obtained from four adjacent sites at 1 mm distance, and the average of the four measurements were analyzed. Upper normal average intima media thickness is estimated to be up to 0.65 mm. Focal thickening >1.5 mm was considered to be a plaque on the wall [18].

### 2.1. Analysis of TNF- $\alpha$ –308G>A (rs1800629) using restriction fragment length polymorphism (RFLP)

About three mL antecubital venous blood sample was collected from the subjects into vacutainer tube containing EDTA and stored at –80 °C. Genomic DNA was extracted from the whole blood (Gene JET Whole Blood Genomic DNA Purification Mini Kit, Germany) according to the manufacturer's instructions. Purified DNA was stored at –20 °C until used. Genomic DNA was amplified using polymerized chain reaction (PCR) with different primers: Forward/Reverse (5'AGGCAATAGGTTTTGAGGGCCAT3')/(5'TCCTCCCTGCTCCGATTCCG3') [19]. The PCR reaction mixture contained 25  $\mu$ l Dream Taq Green in PCR Master Mix (Thermo Scientific, Germany), 15  $\mu$ l of tested DNA, 0.5  $\mu$ M of each primer (Biolegia, BV Nijmegen Netherlands), and nuclease-free water, was added to the PCR mixture to give a final reaction volume of 50  $\mu$ l. As a negative control in each PCR run, nuclease-free water was used instead of the tested DNA. Rapid cyclers PCR (G-Storm Thermal cyclers, England) was used to carry out PCR reactions with the following conditions: 1 cycle of 94 °C for 3 min followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 60 °C for 60 s and extension at 72 °C for 60 s. Finally, an extension step was done at 72 °C for 5 min. If DNA was not used immediately, it was stored at –20 °C till the time of use (Fig. 1). A 10  $\mu$ l PCR product, from each subject was digested with 1  $\mu$ l of restriction enzyme Fast Digest NcoI (Thermo Scientific, Germany), at 37 °C according to the manufacturer's protocol. The digested products were analyzed by 2% agarose gel electrophoresis stained by ethidium bromide and visualized by UV (Fig. 2).

### 2.2. Statistical analysis

Data were presented as mean  $\pm$  SD and range or frequency and percentage. Comparisons between groups were carried out using the  $\chi^2$ -test and Fisher's exact test. The Student's t and the Mann–Whitney were used to test the differences between two groups regarding parametric and nonparametric data, respectively. The one-way analysis of variance (ANOVA) (F) and the Kruskal–Wallis test were used to compare more than two groups as appropriate followed by the post-hoc Bonferroni method to detect differences in pairs. P value < 0.05 was considered significant. All statistical analyses were carried out in STATA/SE version 11.2 for Windows (STATA Corporation, College Station, Texas, USA).

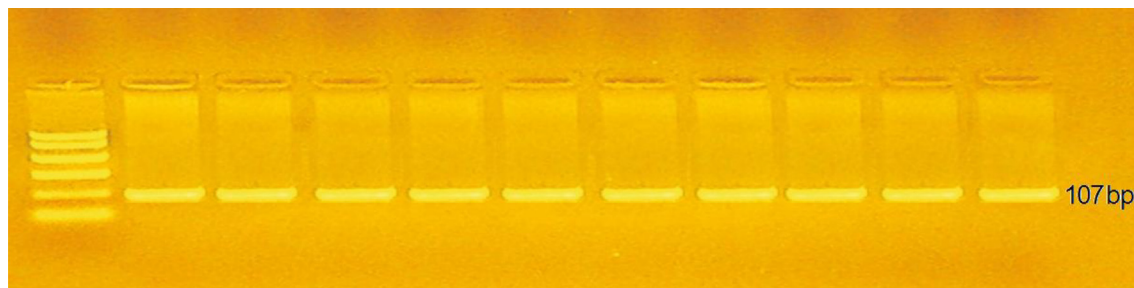
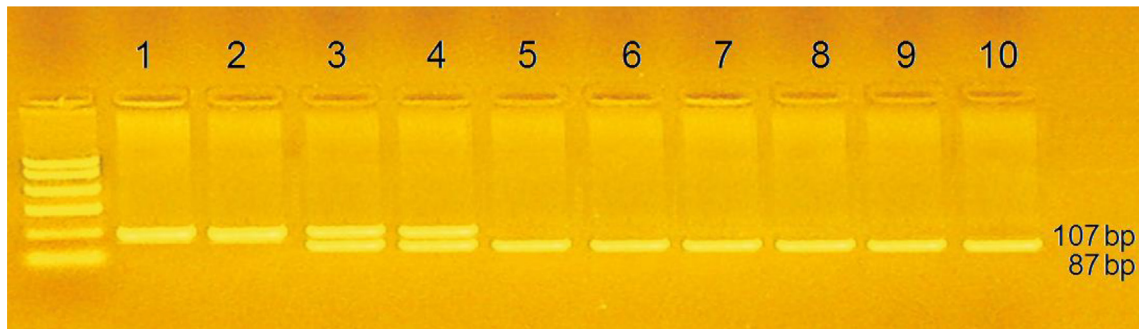


Fig. 1. Ethidium bromide stained gel electrophoresis of TNF- $\alpha$  –308 genome amplified by PCR showing amplicon length of TNF is 107 bp. bp 50–1000 DNA ladder (Gene RullerTm 50 bp DNA Ladder, Thermo Scientific, Germany).



**Fig. 2.** TNF- $\alpha$  –308 G > A allele was distinguished by digestion of the PCR product using Fast Digest NcoI restriction enzyme. Lanes 1 and 2 show AA genotype, lane 3 and 4 show AG genotype and lanes 5–10 show GG genotype.

### 3. Results

There were 50 (28 women and 22 men) RA patients with a mean age of  $41.2 \pm 13.2$  years with a mean disease duration of  $6.1 \pm 4.5$  years and BMI of  $25 \pm 6.2$  together with 40 (23 women and 17 men) age and sex matched healthy controls with a mean age of  $45.4 \pm 10$  years and BMI of  $22.4 \pm 4.2$ . Patients characteristics are presented in Table 1. 18 patients were regularly receiving methotrexate (MTX) (12.5–15 mg/w), 10 were receiving leflunomide (LFN) (20 mg/d), 12 were on regular MTX plus LFN, 8 on sulfasalazine (2gm/d) and 2 received biological treatment; with or without low dose corticosteroids (2.5–10 mg/d) or chloroquine (200 mg/d). NSAIDs were prescribed only when needed. The mean carotid IMT was significantly higher in patients ( $p \leq 0.001$ ) compared to control. The IMT was increased in 23 (46%) of patients

**Table 1**

Laboratory parameters, disease activity score and Larsen's score of rheumatoid arthritis patients.

Variables mean $\pm$ SD (range) or n (%)	RA patients (n = 50)
ESR (mm/1 h)	$43.8 \pm 15.8$ (18–73)
CRP (mg/l)	$12.2 \pm 6.9$ (4–28)
Hemoglobin (g/dl)	$10.8 \pm 1.2$ (9–14)
Platelets ( $10^3$ /ml)	$210.8 \pm 65.2$ (170–480)
WBCs	$6.8 \pm 2.3$
Anti-CCP positive	32 (64)
RF positive	36 (72)
Total cholesterol (mg/dl)	$246.8 \pm 51.7$ (120–340)
LDL (mg/dl)	$156.5 \pm 37.9$ (65–240)
HDL (mg/dl)	$43.6 \pm 26.4$ (40–150)
Triglyceride (mg/dl)	$135.7 \pm 43.1$ (70–255)
DAS 28	$4.1 \pm 0.8$ (3.01–5.64)
Larsen's score	$2.1 \pm 0.9$ (1–4)

RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; WBC: white blood cells; anti-CCP: anti-cyclic citrullinated peptide; RF: rheumatoid factor; LDL: low-density lipoprotein; HDL, high-density lipoprotein, DAS28, disease activity score in 28 joint.

**Table 2**

Carotid ultrasound findings in rheumatoid arthritis patients and control.

Carotid US findings mean $\pm$ SD (range) or n(%)	RA patients (n = 50)	Control (n = 40)	p
IMT (mm)	$0.78 \pm 0.47$ (0.35–1.95)	$0.44 \pm 0.16$ (0.3–1.35)	<b>&lt;0.001</b>
( $\geq 0.65$ mm)	23 (46)	2 (5)	–
Plaques	2 (4)	0 (0)	–
Calcifications	3 (6)	1 (2.5)	–

RA: rheumatoid arthritis; IMT: intima media thickness. Bold values are significant at  $p < 0.05$ .

and 2 (5%) of the control (Table 2). No significant differences ( $p = 0.23$ ) were reported between male and female patients regarding carotid IMT. Allelic and genotypic frequencies of TNF- $\alpha$  –308G>A (rs1800629) gene polymorphisms among controls and patients are shown in Table 3. Table 4 shows that patients with AA genotype had significantly higher DAS-28, lipid profile and increased carotid IMT compared to the other genotypes.

### 4. Discussion

In the last decades data confirmed that RA has been linked strongly to atherosclerosis. Actually, patients diagnosed with RA have greater susceptibility of developments of cardiovascular events. Several common pathomechanisms link RA and atherosclerosis. Inflammation certainly plays a primary role. TNF- $\alpha$  and IL-6 are potent proinflammatory cytokines involved in RA pathogenesis and are independent prognostic markers of consequent CVD. Inflammation in RA changes the concentration of LDL and HDL, consequently accelerating the occurrence of atherosclerosis and CVD events. Alternatively, the increase of oxidative processes, observed frequently in RA, prompts atherosclerosis [20]. Atherosclerosis might be directly related to the primary autoimmune processes, and indirectly by the incidence of metabolic syndrome and reduced physical activity. Moreover, some genetic polymorphisms linked with RA occurrence increase atherosclerosis, although additional polymorphisms do not enhance CVD susceptibility. Also, RA therapies may affect cardiovascular system and atherosclerosis. However, the corner stone of RA management is to control the disease, or achieve remission, which is the most important way to decline the incidence of CVD [21].

In the present study, RA patients were more often carriers of A allele with a higher frequency of TNF- $\alpha$  AA genotype while GG genotype was higher in control. A meta-analysis including 14 studies; 10 European, three Latin American and one Asian, indicated that the TNF- $\alpha$  A allele was significantly associated with RA in

**Table 3**

Allelic and genotypic frequencies of tumor necrosis factor (TNF- $\alpha$ ) polymorphism in rheumatoid arthritis patients and control.

Genotype TNF- $\alpha$ -308 (rs1800629)	RA (n = 50)	Controls (n = 40)
AA	8 (16)	0 (0)
GA	12 (24)	1 (2.5)
GG	30 (60)	39 (97.5)
p	<b>&lt;0.0001</b>	–
Alleles	A	1/80
	G	79/80
p	<b>&lt;0.0001</b>	–

RA: rheumatoid arthritis. Bold values are significant at  $p < 0.05$ .

**Table 4**  
Comparisons among different genotypes in rheumatoid arthritis patients as regards disease activity, laboratory, and radiological data.

Variable mean ± SD	RA patients (n = 50)			P
	AA (n = 8)	GA (n = 12)	GG (n = 30)	
Age (years)	49 ± 11.7	43 ± 16.8	38.4 ± 11.3	0.11
DD (years)	9.9 ± 5.3	3.9 ± 2.9	5 ± 4.3	<b>0.01</b>
DAS28	4.7 ± 0.6	4.4 ± 0.7	3.9 ± 0.8	<b>0.01</b>
Hb (g/dl)	10.5 ± 1.2	10.3 ± 0.8	11.4 ± 2	0.07
WBC ( $\times 10^3/\text{mm}^3$ )	7.01 ± 1.7	6.8 ± 1.6	6.7 ± 1.9	0.92
PLT ( $\times 10^3/\text{mm}^3$ )	370.3 ± 88.3	346.5 ± 77.3	297.4 ± 79.9	<b>0.04</b>
ESR (mm/1 <sup>st</sup> h)	58.3 ± 15.3	46.7 ± 15.7	41.5 ± 16.2	<b>0.01</b>
CRP (mg/dl)	15 ± 8.8	11.7 ± 4.9	11.6 ± 6.9	0.45
Ch (mg/dl)	272.5 ± 62.9	238.3 ± 57.7	204.3 ± 43.6	<b>0.01</b>
LDL (mg/dl)	163.6 ± 15.2	138.9 ± 40	106.1 ± 32.5	0.051
HDL (mg/dl)	38.1 ± 35.8	61.6 ± 34.9	74.7 ± 24.6	<b>0.02</b>
TG (mg/dl)	143.4 ± 58.6	140.1 ± 33.9	125.4 ± 37.9	0.40
Larsen score	2.6 ± 0.5	2.3 ± 1.2	2.13 ± 0.8	0.06
IMT (mm)	1.2 ± 0.4	1.02 ± 0.5	0.5 ± 0.3	<b>&lt;0.001</b>

DD: disease duration; DAS28: disease activity score in 28 joints; Hb: hemoglobin; WBC: white blood cells; PLT: platelets; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Ch: Cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides; IMT: intima media thickness. Bold values are significant at  $p < 0.05$ .

Latin Americans. Conversely, there was no association with the TNF- $\alpha$  A allele in European RA patients [22]. On the contrary, a study that investigated the relationship between TNF- $\alpha$  -308 G polymorphism and the possibility of RA in Hunan's Han Chinese people [23] found that the susceptibility of disease was increased with TNF- $\alpha$  -308 G allele patients, and was more common in females; TNF- $\alpha$  -308 A allele could show a positive role in decreasing the risk of RA in men. Another study [24] proposed that the promoter polymorphism -308 G/A in the TNF- $\alpha$  gene had no significant effect on RA development. These differences in results may be due to the difference in ethnicity, number of patients' and the used PCR technique. Moreover TNF stimulates endothelial damage by the enrollment cells of the immune system, such as the neutrophils, which can facilitate tissue damage. Conversely, anti-TNF therapy has been related with an enhancement of endothelial function in RA patients with severe disease refractory to conventional disease modifying antirheumatic drugs [25].

In this work, patients with IHD or stroke were excluded; a relation between G -308A polymorphism and IHD with an increased risk has been reported [26] and TNF- $\alpha$  was strongly implicated in the pathogenesis of stroke [27]. A meta-analysis evaluated the association between TNF- $\alpha$ G308A gene polymorphism and coronary atherosclerotic heart disease (CHD) risk. Among 35 articles, 17 were related to TNF- $\alpha$  (G308A) gene mutation and CHD but was not significantly associated with CHD risk in Asians [28].

Numerous noninvasive assessments of vascular function and morphology can examine the different stages of subclinical atherosclerosis and provides valuable data on an individual's CVD risk status [21]. In this study, the mean carotid IMT was significantly increased in patients, plaques were present in 2 cases while calcifications tended to be more compared to the control. This may be explained by the development of oxidative modification of LDL, that has been connected to TNF- $\alpha$  action by the activation of superoxide secretion from monocytes and endothelial cells [29]; furthermore, HDL components can be changed by the inflammation, so down their capacity to eliminate cholesterol from atherosclerotic lesions and decreasing their antioxidant action [30]. Also the concentration of LDL and HDL is changed in RA; especially LDLs with small-dense are augmented, while HDLs small-dense are reduced [29], so disturbing the balance to atherosclerosis occurrence.

The genetic background for RA-related atherosclerosis has been identified. Especially, the results demonstrated that rs599839 A/G polymorphism (chromosome 1p13.3) was linked with higher plasma total and LDL cholesterol levels and with an overall enhanced possibility of CVD [20]. The current study reported that

patients with AA genotype had significantly increased carotid IMT compared to the GG genotype which is associated with longer disease duration, high disease activity score, elevated ESR; CRP; and platelet count that are all markers of inflammation. Also RA patients with AA genotype experienced elevated levels of cholesterol and LDL and HDL compared to the GG genotype. Gheita et al. [31] reported that serum TNF- $\alpha$  level was significantly higher in RA patients compared to controls and was significantly higher in those with AA promoter polymorphism.

Among the limitations of this work are the relatively small sample size and not taking into consideration the relation to the used medications.

In conclusion, RA and atherosclerosis are strictly linked to each other; TNF- $\alpha$  -308 A/G polymorphism might increase atherosclerotic susceptibility in RA patients through increased risk of inflammation with subsequent abnormal lipid profile.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

- [1] Bernard C. Progression in early rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2009;23:59–69.
- [2] Liao KP, Karlson EW. Classification and epidemiology of rheumatoid arthritis. In: Hochberg MC, Smolen JS, Weinblatt ME, Weisman MH, editors. *Rheumatology*. Philadelphia, PA: Elsevier; 2011. p. 823–7.
- [3] Rashad NM, Allam RM, Said D, Ali AE, Mohy NM, Abomandour HG. Influence of +299G>A and +62G>A resistin gene promoter variants on cardiovascular risk in Egyptian women with systemic lupus erythematosus. *Egypt Rheumatol* 2019;41(3):215–20.
- [4] Ibrahim SE, Helmi A, Yousef TM, Hassan MS, Farouk N. Association of asymptomatic hyperuricemia and endothelial dysfunction in psoriatic arthritis. *Egypt Rheumatol* 2012;34(2):83–9.
- [5] Bader El-Din A, El-Bakry SA, Abdel Zaher A, Alhassanein KF, Al-Dakrony AM, Sayed S, et al. Determinants of atherosclerosis in an Egyptian cohort of systemic sclerosis: relation to disease activity and severity. *Egypt Rheumatol* 2016;38(3):195–201.
- [6] El-Najjar AR, Elsammak AA. Association between serum homocysteine and arterial stiffness in patients with Behçet's disease. *Egypt Rheumatol* 2019. epub ahead of print.

- [7] El-Menoufy MA, El-Kak AA, Ahmed MA. Unusual CD4+CD28<sup>-</sup> T lymphocyte subset is implicated in the pathogenesis of early atherosclerosis in patients with rheumatoid arthritis. *Egypt Rheumatol* 2019;41(2):105–9.
- [8] Ozisler C, Atis A, Karaaslan Y, Elalmis OU, Parlak IS, Dortbas F, et al. Clinical significance of aortic stiffness, carotid intima-media thickness and serum osteoprotegerin level in rheumatoid arthritis patients. *Egypt Rheumatol* 2019;41(2):111–5.
- [9] Choy EHS, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001;344:907–16.
- [10] Kassem E, Ghonimy R, Adel M, El-Sharnoby G. Non traditional risk factors of carotid atherosclerosis in rheumatoid arthritis. *Egypt Rheumatol* 2011;33(3):113–9.
- [11] Gontsaruyk DA. Indicators proinflammatory cytokines tnf- $\alpha$  in patients with chronic pancreatitis and coronary heart disease. *Pancreatol* 2012;12:519–20.
- [12] Ahmad J, Ahmed F, Siddiqui MA, Khan AA, Katyal B, Hameed B, et al. Inflammatory markers, insulin resistance and carotid intima-media thickness in North-Indian type 2diabetic subjects. *J Assoc Phys India* 2007;55:693.
- [13] Aguilón JC, Cruzat A, Aravena O, Salazar L, Llanos C, Cuchacovich M. Could single-nucleotide polymorphisms (SNPs) affecting the tumor necrosis factor promoter be considered as part of rheumatoid arthritis evolution? *Immunobiology* 2006;211:75–84.
- [14] Cuenca J, Cuchacovich M, Pérez C, Ferreira L, Aguirre A, Schiattino I, et al. The –308 polymorphism in the tumor necrosis factor (TNF) gene promoter region and ex vivo lipopolysaccharide-induced TNF expression and cytotoxic activity in Chilean patients with rheumatoid arthritis. *Rheumatology (Oxford)* 2003;42:308–13.
- [15] Jiménez-Morales S, Velázquez-Cruz R, Ramírez-Bello J, Bonilla-González E, Romero-Hidalgo S, Escamilla-Guerrero G, et al. Tumor necrosis factor-alpha is a common genetic risk factor for asthma, juvenile rheumatoidarthritis, and systemic lupus erythematosus in a Mexican pediatricpopulation. *Hum Immunol* 2009;70:251–6.
- [16] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
- [17] Prevo MLL, Vanthof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores tCChat include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44–8.
- [18] Celik S, Erdogan T, Kasap H, Kaplan S, Durmuş I, Gedik O, Kiriş A. Carotid intima-media thickness in patients with isolated coronary artery ectasia. *Atherosclerosis* 2007;190:385–7.
- [19] Al-Rayes H, Al-Swailem R, Albelawi M, Arfin M, Al-Asmari A, Tariq M. TNF- $\alpha$  and TNF- $\beta$  gene polymorphism in saudi rheumatoid arthritis patients. *Clin Med Insights Arthritis Musculoskelet Disord* 2011;4:55–63.
- [20] Lorenzo C, Nicola B, Giovanni C, Flora I, Vittorio G, Roberto C. Atherosclerosis and rheumatoid arthritis: more than a simple association. *Mediat Inflamm* 2012;2012:1–8. Article ID 147354.
- [21] Mirjafari H, Al-Husain A, Bruce IN. Cardiovascular risk factors in inflammatory arthritis. *Curr Opin Lipidol* 2011;22(4):296–301.
- [22] Lee YH, Ji JD, Song GG. Tumor necrosis factor-alpha promoter -308 A/G polymorphism and rheumatoid arthritis susceptibility: a metaanalysis. *J Rheumatol* 2007;34(1):43–9.
- [23] Li F, Xie X, Chen J, Gao J, Lu F. Association of TNF- $\alpha$  gene polymorphisms with the risk of rheumatoid arthritis in Han Chinese population from Hunan. *Zhong Nan Da XueXueBao Yi Xue Ban* 2015;40:945–54.
- [24] Manolova I, Ivanova M, Stoilov R, Rashkov R, Stanilova S. Association of single nucleotide polymorphism at position –308 of the tumor necrosis factor-alpha gene with ankylosing spondylitis and rheumatoid arthritis. *Biotechnol Biotechnol Equip* 2014;28:1108–14.
- [25] Welsh P, Woodward M, Rumley A, Lowe G. Associations of plasma proinflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. *Br J Haematol* 2008;141:852–61.
- [26] Wang J, He Y, Yang Y, Song T, Chen N, Zhou Y. Association between the TNF- $\alpha$  G –308A polymorphism and risk of ischemic heart disease: a metaanalysis. *Int J Clin Exp Med* 2015;8:8880–92.
- [27] Rodríguez-Carreón AA, Zúñiga J, Hernández-Pacheco G, Rodríguez-Pérez JM, Pérez-Hernández N, Montes de Oca JV, et al. Tumor necrosis factor-alpha –308 promoter polymorphism contributes independently to HLA alleles in the severity of rheumatoid arthritis in Mexicans. *J Autoimmun* 2005;24:63.
- [28] Yuepeng J, Zhao X, Zhao Y, Li L. Gene polymorphism associated with TNF- $\alpha$  (G308A) IL-6 (C174G) and susceptibility to coronary atherosclerotic heart disease: a meta-analysis. *Med (Baltimore)* 2019;98(2):e13813.
- [29] Ku IA, Imboden JB, Hsue PY, Ganz P. Rheumatoid arthritis—a model of systemic inflammation driving atherosclerosis. *Circulation J* 2009;73(6):977–85.
- [30] Van Lenten BJ, Reddy ST, Navab M, Fogelman AM. Understanding changes in high density lipoproteins during the acute phase response. *Arteriosclerosis Thromb Vasc Biol* 2006;26(8):1687–8.
- [31] Gheita TA, Azkalany GS, Gaber W, Mohey A. Clinical significance of serum TNF $\alpha$  and –308 G/A promoter polymorphism in rheumatoid arthritis. *Egypt Rheumatol* 2015;37:49–54.