Original Article

Tumor necrosis factor-alpha −308G/A gene polymorphism and novel biomarker profiles in patients with Takayasu arteritis

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ABSTRACT

Background: Takayasu arteritis (TA) is an idiopathic chronic inflammatory disease of the aorta and its branches, leading to stenosis, occlusion, and aneurysmal dilatation. Tumor necrosis factor-alpha (TNF-α) is a cytokine with pleomorphic actions and plays a pivotal role in inflammation; the serum level of TNF-α is genetically determined. However, the literature lacks adequate information on the association of TNF-α polymorphisms with TA. Hence, the present study investigates the contribution of TNF-α polymorphism toward the complex etiology of TA.
Methods: A cross-sectional study was performed in 87 patients with TA and 90 controls. A promoter region polymorphism of TNF-α, rs1800629 G/A, or −308G/A was genotyped in all the study subjects followed by a case−control association study. Furthermore, to understand the biomarker profile, levels of specific markers such as erythrocyte sedimentation rate, serum high-sensitivity C-reactive protein, interleukin-18, interleukin-6, and TNF-α were measured in all the study subjects.
Results: All the inflammatory markers were significantly higher in the TA patients than in the controls. The genetic study (available for 57 TA patients and 36 controls) revealed that the TNF-α −308A allele was overrepresented in the TA patients (12% vs 7%). The TNF-α −308A allele correlated with the increased TNF-α levels, but it could not attain significance because of a small sample size.
Conclusion: The TNF-α −308G/A polymorphism is associated with TNF-α levels in Indian population, which might have implications for clinical risk stratification and treatment. The different TNF-α gene promoter polymorphism might contribute to the molecular pathogenesis of TA. However, further study of the underlying mechanism is warranted.

1. Introduction

Takayasu arteritis (TA) is an idiopathic chronic inflammatory disease of the aorta and its branches, leading to stenosis, occlusion, and aneurysmal dilatation.[1] TA is the most common cause of renovascular hypertension (HTN) in India and other Southeast Asian countries. TA predominantly affects women in the 2nd to 3rd decade of life.[2] Incidence reported in India is nearly 150 patients per million people per year.[3,4]

Tumor necrosis factor (TNF) is a cytokine with pleomorphic actions and plays a pivotal role in inflammation and host defense against infections.[3] The serum level of TNF-α varies from individual

to individual and is genetically determined.[3] The gene for TNF-α is located within the major histocompatibility complex region on chromosome 6p21, which is a highly polymorphic region, and genetic evidence for TA prevails in the HLA region.[5,6] There are many biallelic single-nucleotide polymorphisms (SNPs) in and around the TNF-α gene. One such polymorphism, rs1800629 G/A or −308G/A, is located upstream of the TNF-α gene, known to influence TNF-α levels.[7,8] As compared with the TNF-α −308 G allele, the A allele has higher transcriptional activity, resulting in a higher serum TNF-α level.[8]

TNF-α −308G/A promoter region gene polymorphism has been reported to be associated with several autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis and infections such as tuberculosis.[9] Association of TNF polymorphisms has not been well studied with TA. Lv et al reported the lack of association between polymorphisms of the TNF-α promoter gene

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with TA in Chinese population.[10] A small study failed to establish a specific association between the polymorphism and TA in South Indian patients.[11] However, the literature lacks adequate information on the association of TNF-α polymorphisms with TA in north Indians.[12] Taking into consideration the probable role of TNF-α in TA, the present study was designed to analyze the TNF-α promoter region −308G/A polymorphism in TA patients and study its relationship with the severity of the disease in north Indian patients.

2. Materials and methods

2.1. Study groups

A cross-sectional study was performed in 87 patients with TA and 90 controls. The study groups were age and gender matched. All patients underwent a detailed clinical assessment of symptoms of disease activity and symptoms and signs of vascular insufficiencies such as claudication, differences in blood pressure (BP) among the limbs, pulse inequalities, and bruits. Disease activity was assessed according to the National Institutes of Health criteria for the active disease.

Diagnosis of TA in such patients was made clinically with support of vascular imaging, and classification of the disease was done as per ACR criteria[13]:

1. Development of symptoms or findings related to TA at age ≤40 years.
2. Development and worsening of fatigue and discomfort in muscles of one or more extremities while in use, especially the upper extremities.
3. Decreased pulsation of one or both brachial arteries.
4. A difference of >10 mmHg in systolic BP between arms.
5. Bruit audible on auscultation over one or both subclavian arteries or abdominal aorta.
6. Arteriographic narrowing or occlusion of the entire aorta, its primary branches, or large arteries in the proximal upper or lower extremities, not due to arteriosclerosis, fibromuscular dysplasia, or similar causes; changes usually focal or segmental.

At least three of these six criteria were to be fulfilled to confirm the diagnosis of TA. Patients with the creatinine level of >1.5 mg/dl, hemodynamic instability, congestive heart failure, pregnancy, systemic inflammatory disorders, and connective tissue disorders were excluded.

Criteria for active disease in patients with TA[14] included the following:

1. Systemic features, such as fever and musculoskeletal pain (no other cause identified).
2. Elevated erythrocyte sedimentation rate (ESR).
3. Features of vascular ischemia or inflammation, such as claudication, diminished or absent pulse, bruit, vascular pain (carotidynia), and asymmetric BP in either upper or lower limbs (or both).

New onset or worsening of two or more features indicates "active disease."

Angiographic classification of the pattern of disease will be done according to the classification by Hata et al.[15]

2.2. Sample collection

Five to eight milliliters of blood was drawn in acid citrate dextrose (ACD) anticoagulant. Two to three milliliters of blood was transferred to another vial and clotted to collect serum for estimation of biochemical parameters. The remaining blood sample

was centrifuged (A-4-62; Eppendorf, Hamburg, Germany) for 10 min at 1500 rpm at 4°C for plasma extraction. The latter was also used for the analyses of biochemical parameters. Peripheral blood leucocytes were used for DNA extraction by modified salting out procedure reported by Miller et al, 1988.[16]

2.3. Genotyping

All the 87 TA patients and the 90 controls have been sampled, but DNA extraction was successful for only 57 TA patients and 36 controls. The genomic loci spanning TNF-α −308G/A polymorphism was amplified by polymerase chain reaction (PCR) using forward, 5′-GCT GAA CCC CGT CC-3′, and reverse, 5′-CGA CTT GGG GCA GG-3′, primers. Twenty microliters of the reaction mixture contained 50 ng of DNA, 3 pmol of each primer, 1X Taq DNA polymerase buffer, 0.33 U of Taq DNA polymerase enzyme, and 0.2 mmol/l of deoxynucleotide triphosphates. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 38 cycles of denaturation at 95°C for 40 s, annealing at 56°C for 30 s and extension at 72 °C for 45 s, followed by a final extension at 72°C of 10 min. The PCR product was purified by polyethylene glycol purification and was subjected to genotyping by using a Snapshot multiplex kit (Applied Biosystems, Foster city, California, USA). The Snapshot primer sequence was 5′-GCT GAA CCC CGT CC-3′ (annealing at 56°C). PerlPrimer was used to design all the primers. Peak Scanner Software v1.0 was used to read peaks for allele determination.

2.4. Biomarker estimation

The study included inflammatory markers assessment for disease activity in TA. Specific markers of activity included ESR and serum high-sensitivity C-reactive protein (hs-CRP), interleukin-18 (IL-18), interleukin-6 (IL-6), and TNF-α levels. The TNF-α levels were estimated using an enzymatic colorimetry kit (Roche Diagnostics GmbH, Manheim, Germany). Enzyme-linked immunosorbent assay was used to determine the levels of IL-6 (Diaclone SAS, Besancon, France) and IL-18 (Roche Diagnostics GmbH, Manheim, Germany) on a Cobas c501 autoanalyzer (Roche Diagnostics GmbH, Manheim, Germany).

2.5. Biostatistical analyses

A goodness-of-fit test was performed for testing the Hardy−Weinberg equilibrium (HWE), and a χ^2 test compared the genotype and allele frequencies of −308G/A TNF-α polymorphism among the two groups. The power of the sample size to detect the association at $\alpha = 0.05$ was calculated using EPIINFO ver.6 software. SPSS 12 (SPSS Inc., Chicago, Illinois, USA) and EPIINFO ver.6 (Centers for Disease Control, Atlanta, Georgia, USA) software were used for statistical analyses. The genotype and allele distribution were compared among the two groups using the multiple logistic regression analysis. The biochemical parameters were expressed as mean ± standard deviation. The levels between TA cases and healthy controls were analyzed with the general linear model after adjustment for confounding factors of age, gender, and body mass index. Pearson's correlation was performed using SPSS 12 to estimate correlation among the studied biomarkers. A p value of ≤0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

The study subjects included a total of 177 consecutive north Indian unrelated participants comprising 90 healthy controls from

general public and 87 TA patients. The age at presentation ranged from 7 years to 48 years with the mean age of 25.7 years. Out of 87 patients, 65 (75%) were female and 22 (25%) were male, yielding a female to male ratio of 3:1.

Limb claudication was the most common presenting symptom seen in 49 patients (56.3%). Neck pain or carotidynia was present in 21 patients (24.1%). Headache was reported by 17 patients (19.5%). Syncope was a presenting symptom in 15 patients (17.2%). A total of 23 patients (26.4%) complained of dyspnea. This was attributed to diastolic dysfunction in 11 patients (47.8%), severe systolic dysfunction (left ventricular ejection fraction < 30%) in 5 patients (21.7%), and moderate or severe aortic regurgitation in 5 patients (21.7%). The exact cause for dyspnea was not known in 2 patients. Chest pain was the presenting complaint in 4 patients (4.5%). Right carotid pulse was absent or feeble in 69 patients (79.3%). Left carotid pulse was absent or feeble in 62 patients (71.2%). Right upper limb pulses (brachial and radial) were feeble or absent in 26 patients (29.8%), and left upper limb pulses (brachial and radial) were absent or feeble in 30 patients (34.4%). Demographic and clinical characteristics were similar in patients in whom DNA extraction was successful.

3.2. Pattern of aortic involvement

The pattern of aortic involvement was assessed using computed tomography (CT) angiography or conventional angiography. In our study, the most common pattern of involvement was the Type III pattern seen in 19 (21.8%) patients, followed by Type IIa (18 patients, 20%), Type V (18 patients, 20%), and Type IV pattern (16 patients, 18%).

3.3. Genetic study

3.3.1. Genomic DNA isolation
DNA isolation was performed for all the subjects. Agarose gel of 0.8% was used to analyze the genomic DNA. Genotyping was performed in samples with good-quality DNA; the sample comprised 57 TA patients and 36 healthy controls.

3.3.2. Genetic analyses
The TNF-α −308G/A genotype was deduced and assigned to the study subjects based on the data obtained from SnapShot genotyping. The goodness-of-fit test showed HWE harmony between the observed and expected genotypes frequencies ($p > 0.05$) in both the study groups.

3.3.3. Single locus analyses
The genotypic and allelic frequencies of TNF-α variation were compared among the two groups, and the same is summarized in Table 1. The multiple logistic regression analysis did not reveal any significant association of allele or genotypes with TA susceptibility. The −308A allele was overrepresented in the TA patients. It was seen in 12.3% of the cases, as compared with 7.0% of the control subjects. Nevertheless, the difference could not be significant due to the inadequate sample size.

3.3.4. Biochemical analysis
The serum levels of the studied biomarkers, namely ESR, CRP, TNF-α, IL-6, and IL-18, levels are summarized. All the inflammatory biomarkers were consistently higher in the cases than the controls. Furthermore, all the inflammatory biomarkers were consistently higher in the cases in all subtypes of TA than in the controls (Supplementary Table 1).

3.4. Correlation analyses within biomarkers

Correlation analyses among the studied biomarkers revealed significant positive correlation between ESR and CRP levels in cases ($p < 0.05$). However, none of the inflammatory markers depicted significant correlation in TA patients as well as in healthy controls (Table 2).

3.5. Correlation analyses −308G/A TNF-α genotypes and biomarkers

Levels of determined biomarkers i.e. ESR, CRP, TNF-α, IL-6, and IL-18 were assessed in each genotype in both the study groups i.e. controls and patients, and the levels were compared on the basis of genotype (Table 3). The comparison included only those subjects who had the complete data of biomarker level and genotype; hence, the analyses included 57 TA patients and 35 controls. The TNF-α levels showed an increasing trend in TA patients with increasing A allele; however, no significance was achieved. Any particular trend was not observed in other biomarkers.

4. Discussion

The present study investigated the role of TNF-α −308G/A polymorphism in TA patients and its influence on various inflammatory biomarkers in north Indian study subjects. Furthermore, the disease activity was evaluated by assessing the clinical symptoms and conventional markers of inflammation such as ESR and

Table 1
Genotype and allele distribution of the TNF-α polymorphism −308G/A (or rs1800629 G/A in Takayasu arteritis patients and healthy controls.

TNF-α	Genetic model	Genotype/Allele	Controls ($n = 36$)	Patients ($n = 57$)	p-value	Chi-square	OR (95% CI)
−308G/A (or rs1800629 G/A)	Codominant	GG	31 (86.1%)	45 (79.0%)	Reference	—	—
		GA	5 (13.9%)	10 (17.5%)	0.36	0.85	1.79 (0.52−6.16)
		AA	0 (0%)	2 (3.5%)	[a]	—	—
	Log-additive	G	67 (93%)	100 (87.7%)	Reference	—	—
		A	5 (7%)	14 (12.3%)	0.09	2.83	2.64 (0.85−8.17)
	Dominant	GG	31 (86.1%)	45 (79.0%)	Reference	—	—
		GA + AA	5 (13.9%)	12 (21.0%)	0.18	1.78	2.30 (0.68−7.86)
	Recessive	GG + GA	36 (100%)	55 (96.5%)	Reference	—	—
		AA	0 (0%)	2 (3.5%)	[a]	—	—
	Over dominant	GG + AA	31 (86.1%)	47 (82.5%)	Reference	—	—
		GA	5 (13.9%)	10 (17.5%)	0.46	0.54	1.57 (0.47−5.29)

p-values were obtained after adjustment with age, sex, and BMI by multivariate logistic regression analysis using SPSS 15.0 software. The allele frequency was compared by χ^2 test.

n, number; %, percent distribution; OR, odds ratio; CI, confidence interval; TNF-α, tumor necrosis factor-alpha levels.

[a] p-value cannot be calculated as TT genotype was not observed in the control group.

Table 2
Correlation analysis between biomarkers in TA patients and healthy controls.

ESR	$r = 1$			**Controls**	
CRP	$r = 0.38$ $p = 0.32$	$r = 1$			
TNF-α	$r = 0.177$ $p = 0.101$	$r = -0.041$ $p = 0.704$	$r = 1$		
IL-6	$r = -0.164$ $p = 0.130$	$r = -0.108$ $p = 0.321$	$r = 0.114$ $p = 0.293$	$r = 1$	
IL-18	$r = 0.017$ $p = 0.879$	$r = 0.041$ $p = 0.708$	$r = 0.042$ $p = 0.702$	$r = -0.129$ $p = 0.233$	$r = 1$
Biomarkers	**ESR**	**CRP**	TNF-α	**IL-6**	**IL-18**
ESR	$r = 1$			**TA patients**	
CRP	$r = 0.73$ $p = 0.03$	$r = 1$			
TNF-α	$r = 0.093$ $p = 0.398$	$r = -0.015$ $p = 0.890$	$r = 1$		
IL-6	$r = -0.127$ $p = 0.250$	$r = -0.064$ $p = 0.561$	$r = 0.036$ $p = 0.745$	$r = 1$	
IL-18	$r = -0.135$ $p = 0.219$	$r = -0.020$ $p = 0.219$	$r = 0.055$ $p = 0.618$	$r = -0.035$ $p = 0.754$	$r = 1$
Biomarkers	**ESR**	**CRP**	**TNF alpha**	**IL-6**	**IL-18**

TA, Takayasu arteritis; ESR, erythrocyte sedimentation rate; hs-CRP, serum high-sensitivity C-reactive protein; IL-18, interleukin-18; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha levels; P, *p*-value. $p \leq 0.05$ was used as cutoff for significance.

hs-CRP, followed by newer markers of inflammation, i.e., TNF-α, IL-18, and IL-6 levels. The preliminary results reveal an over-representation of TNF-α −308A allele in TA. The positive correlation trend of TNF-α −308A allele with increasing TNF-α levels in TA patients strengthened our findings of the involvement of TNF-α −308G/A polymorphism in disease pathophysiology.

The overrepresentation of females in TA patients was comparable to previous studies conducted on Indian and other Asian populations.[12,13,17,18] The most common clinical symptom of presentation of our patients was claudication. Only 20% of patients were diagnosed on the basis of new-onset HTN. However, our findings are in contrast to those of the previous studies that report that the most common mode of presentation in India and Japan was HTN (50% and 55%, respectively), followed by claudication (25% and 35%, respectively).[12,18] This may be attributed to the fact that most of the patients seen at our center are having advanced disease with extensive arch vessel involvement. Owing to the significant subclavian involvement, patients have claudication commonly, and cuff BP is not reflective of the actual intraarterial BP. This is further supported by the fact that in our series, 30 patients have an absent left radial pulse and 26 patients had an absent right radial pulse. Also, the pattern of aortic involvement assessed using CT

angiography revealed the Type III pattern as the most common one in our study patients.

The role of genetic predisposition in the susceptibility for TA has been investigated worldwide[19,20]; however, the literature lacks reports for larger sample size studies in Indian populations.[17] The studied SNP, TNF-α −308G/A, followed HWE in both study cases and controls. The genetic analyses results revealed an over-representation of −308A allele in TA patients. In both log-additive and dominant model, the TNF-α −308A allele is represented by ~1.5 times higher in TA patients than in the controls. Nevertheless, larger sample size would be required to affirm the genetic effect correctly and exemplify the association of the risk genotype or allele with TA susceptibility.

The unreliability of ESR and hs-CRP in TA has prompted the search for a more reliable serological marker of disease activity. Hence, in the present study, both conventional and newer bio-markers were studied to provide significant insights into the mechanism of the disease pathophysiology. Interestingly, the biomarker analysis suggests that individuals with TA have significantly higher levels of the inflammatory markers than the controls. The inflammatory markers were also studied in the different subset of patients defined on the basis of the pattern of aortic involvement; however, the results were not significant.

Furthermore, we performed correlation analyses among the biomarkers to get a glimpse of individual and interactive effects of these markers on the phenotypic outcome. The study revealed that the ESR and CRP levels correlated very well across the spectrum of the disease. ESR has a sensitivity and specificity for active TA of 72% and 56%, whereas CRP has a sensitivity of 71.4% and specificity up to 100% for active disease.[21] ESR and CRP, being markers of general inflammation, were expected to be correlated with disease activity. However, the new biomarkers (TNF-α, IL-6, and IL-18) appear not to be correlating with each other or with ESR and CRP. Correlation of CRP with IL-6 is expected due to the fact that CRP is synthesized in the liver in response to IL-6. Rest of the biomarkers, i.e. TNF-α and IL-18, represent two separate pathways of inflammation. So, although their levels are raised individually in cases, levels may not be correlating as these two pathways of inflammation are mutually exclusive. Also, the pattern of elevation of an inflammatory marker was not predictive of the pattern of vascular involvement. This can be explained by the fact that ESR and CRP are non-specific markers of inflammation, indicating only a systemic inflammatory state. TNF-α, IL-6, and IL-18 also represent different pathways of inflammation, rather than an anatomical localization. Similar to previous studies, we also observed the significant increase of TNF-α level in patients with active TA. The results suggest that TNF-α might be a useful marker for monitoring the activity of TA. Because it does not

Table 3
Correlation analysis of studied biomarker levels in subjects with TNF-α polymorphism −308G/A genotyped in TA patients and healthy controls.

Biochemical markers (units)	Controls[a] ($n = 35$)		TA patients ($n = 57$)		
	GG ($n = 30$)	GA ($n = 5$)	GG ($n = 45$)	GA ($n = 10$)	AA ($n = 2$)
ESR (mm/hr)	12.07 ± 4.63 Reference	9.60 ± 4.04 $p = 0.26$	37.50 ± 14.17 Reference	27.52 ± 11.51 $p = 0.13$	61.51 ± 23.38 $p = 0.42$
CRP (mg/dl)	2.59 ± 0.94 Reference	2.34 ± 0.50 $p = 0.50$	7.01 ± 1.52 Reference	6.33 ± 2.17 $p = 0.47$	6.72 ± 0.13 $p = 0.91$
TNFα (pg/ml)	10.64 ± 3.03 Reference	9.91 ± 3.62 $p = 0.64$	19.41 ± 3.26 Reference	19.65 ± 3.73 $p = 0.80$	21.55 ± 4.99 $p = 0.36$
IL-6 (pg/ml)	9.04 ± 3.22 Reference	11.01 ± 3.72 $p = 0.22$	21.43 ± 4.24 Reference	19.67 ± 4.58 $p = 0.26$	16.95 ± 4.16 $p = 0.16$
IL-18 (pg/ml)	10.31 ± 2.56 Reference	11.52 ± 2.14 $p = 0.33$	21.52 ± 4.40 Reference	23.23 ± 4.72 $p = 0.28$	22.47 ± 10.37 $p = 0.78$

TA, Takayasu arteritis; ESR, erythrocyte sedimentation rate; hs-CRP, serum high-sensitivity C-reactive protein; IL-18, interleukin-18; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha levels; P, *p*-value. $p \leq 0.05$ was used as cutoff for significance.

[a] The control group did not have any subject with −308AA genotype. The comparison of means was done within a group using EPIINFO. n, number of samples; *p*, *p*-value.

correlate with ESR and CRP levels, it might prove to be a useful biomarker in patients with active disease and normal ESR and CRP levels. In our study, the patients with active disease, as indicated by the clinical profile, ESR and CRP experienced significant increases in TNF-α plasma level compared with normal healthy controls. Further larger sample studies are required, and these serological markers show promise as future biomarkers for TA.

To strengthen the genetic outcome, we investigated the possible correlation of the −308G/A with inflammatory biomarkers. Although no significant correlation was found between the genotype and level of inflammatory markers in cases or controls, the plasma levels of TNF-α in patient with GG, GA, and AA genotype was 21.55 ± 4.99 pg/ml, 19.65 ± 3.73 pg/ml, and 19.41 ± 3.26 pg/ml, respectively. This difference depicted a precise trend of increasing TNF-α levels with increasing risk allele. Also, in previous studies conducted by Wilson et al[5] and Braun et al,[4] it has been shown that TNF-α −308G allele is associated with lesser TNF-α production than A allele, suggesting that this polymorphism might affect TNF-α expression at the transcriptional level and contribute to the outcome of TA.

To conclude, the results suggest that TNF-α plays a key role in the pathophysiology of TA. The traditional medical treatments for patients with active TA includes immunosuppressive agents such as corticosteroids and methotrexate. It may be apt to hypothesize that carriers of the −308 GG and GA genotype with lower TNF-α production might respond better to medical treatment. Anti-TNF-α agents have been proven effective in the treatment of patients with TA, especially in refractory cases with high TNF-α levels. High TNF-α production might be associated with better responses to anti-TNF-α therapy. Identifying patients with genotypes predisposed toward higher expression levels of TNF-α could be useful for early risk stratification and treatment of TA. The present study, however, did not study the effect of drugs in relation to polymorphisms. These findings need to be confirmed in further studies.

5.1. Limitations of the study

The study has many limitations. First, our findings, which are based on a relatively large population of patients with TA, have not been confirmed in a second independent cohort, and the results await replication in different populations. Second, in vitro experiments are required to investigate the underlying mechanism of −308G/A polymorphism in TNF-α production. Third, all the patients have been recruited from a tertiary care center; hence referral bias cannot be ruled out.

5. Conclusion

TNF-α plasma levels are elevated in the active stage of TA, which may be a key cytokine in TA development and progression. The −308G/A polymorphism is associated with decreased TNF-α expression in Indian population, which might have implications for clinical risk stratification. The different TNF-α gene promoter polymorphism haplotypes might contribute to the molecular pathogenesis of TA. The haplotype analysis could provide further insight into the role of the TNF-α promoter in the molecular pathogenesis of TA. Therefore, further study of the underlying mechanism is warranted.

5.1. Key messages

- What is already known about this subject?

Tumor necrosis factor (TNF) is a cytokine with pleomorphic actions and plays a pivotal role in inflammation and host defense

against infections. The serum level of TNF-α varies from individual to individual and is genetically determined. There are many biallelic single-nucleotide polymorphisms (SNPs) in and around the TNF-α gene. One such G/A polymorphism is located upstream of gene at −308 and is known to influence TNF-α levels. As compared with the - TNF-α −308 G allele, A allele has higher transcriptional activity resulting in a higher serum TNF-α level. TNF-α - 308 promoter gene polymorphism has been reported to be associated with several autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, and infections such as tuberculosis. There was no previous study on TNF-α gene polymorphism in TA patients in north Indian population.

- What does this study add?

This study was planned to analyze TNF-α promoter region −308 polymorphism in TA patients and study its relationship to the severity of the disease in north Indian patients. Results show that the frequency of the −308 GA genotype and A allele is nonsignificantly higher among patients with TA. Furthermore, carriers of the −308 GA and AA genotype had nonsignificantly higher TNF-α levels than carriers of −308 GG genotype, suggesting that this polymorphism might affect TNF-α expression at the transcriptional level and contribute to the outcome of TA. In our study, the patients with active disease, as indicated by clinical profile, ESR, and CRP, experienced significant increases in TNF-α plasma level compared with normal healthy controls. The results suggest that TNF-α plays a key role in the pathophysiology of TA.

- How might this impact on clinical practice?

The traditional medical treatments for patients with active TA include immunosuppressive agents such as corticosteroids and methotrexate. It may be apt to hypothesize that the carriers of −308 GG or GA genotype, with lower TNF-α production, might respond better to medical treatment. Anti-TNF-α agents have been proven effective in the treatment of patients with TA, especially in refractory cases with high TNF-α levels. High TNF-α production might be associated with better responses to anti-TNF-α therapy. Identifying patients with genotypes predisposed toward higher expression levels of TNF-α could be useful for early risk stratification and treatment of TA. However, the present study did not study the effect of drugs in relation to polymorphisms. These findings need to be confirmed in further studies. Further studies are therefore required.

Conflicts of interest

All authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ihj.2018.09.004.

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