ORIGINAL ARTICLE

ASSOCIATION OF TUMOUR NECROSIS FACTOR-ALPHA -308 G/A PROMOTER POLYMORPHISM WITH SUSCEPTIBILITY AND DISEASE PROFILE OF RHEUMATOID ARTHRITIS

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Background: Single nucleotide polymorphism underlying the auto-immune process governing the pathologic manifestations of rheumatoid arthritis has been the focus of study for quite a while. TNF-alpha -308 G/A promoter polymorphism have been reported to be responsible for a number of manifestations of rheumatoid arthritis. **Methods**: This case-control study was conducted at the department of Rheumatology at Pakistan Institute of Medical Sciences Islamabad from 9th May to 9th August 2019 with a focus to determine the Association of tumour necrosis factor-alpha -308 G/A promoter polymorphism with susceptibility and disease profile of rheumatoid arthritis. One hundred and fifty cases with diagnosed rheumatoid arthritis and 150 age and gender matched controls were enrolled in the study. Their genotyping was done for tumour necrosis factor-alpha -308 G/A promoter polymorphism. **Results**: The genotypic analysis showed that GG genotype was the most common genotype found in 118 cases (78.66%) followed by GA (18.66%) and AA genotype (2.6%) p=0.0096 in both cases and controls. Overall, G allele was more common than A in both cases and controls pointing towards the preponderance of G genotype in our population. (p=0.003). However, the GA genotype and A allelotype was more common among cases with rheumatoid arthritis (p <0.05). No significant association of G/A polymorphism with smoking and gender, however, within gender, males had a significantly more expression of the GA genotype and A allelotype (p <0.05). **Conclusion**: There is a significantly more expression of the GA genotype and the A allelotype of the TNF-alpha -308 G/A promoter gene in rheumatoid arthritis patients in our population. Similarly, more males, compared to females have increased expression of the GA genotype as well as the A allelotype.

Keywords: Rheumatoid Arthritis; Tumour necrosis factor alpha; Auto-immune disease; Single nucleotide polymorphism; Allelotype

Citation: Khurshid S, Zeb A, Bano S, Rasheed U, Zammurrad S, Khan MS *et al*. Association of tumour necrosis factor-alpha -308 G/A promoter polymorphism with susceptibility and disease profile of rheumatoid arthritis. J Ayub Med Coll Abbottabad 2020;32(2):184–8.

INTRODUCTION

The hallmark of rheumatoid arthritis, an auto-immune disease, is destruction of bones secondary to chronic inflammation of the synovial membranes covering the joint surfaces leading to loss of joint structures and function.[1–3] The overall prevalence of rheumatoid arthritis ranges between 0.5–1% worldwide with population-specific variations in different parts of the world.[4] Females are affected by rheumatoid arthritis more than males as depicted by the male: female ratio of 1:2–3. Though rheumatoid arthritis typically develops in the age bracket of 30–55 years, it can manifest at any age.[5] In Pakistan, the prevalence of rheumatoid arthritis varies according to the geographic presence. For example, the prevalence of rheumatoid arthritis in northern part of country is 0.55% while in southern part of the country, the prevalence of rheumatoid arthritis is 0.142%.[6]

Although the exact cause of rheumatoid arthritis is not yet known[7], a number of environmental and genetic factors have been identified which are believed to play a defining role in the rheumatoid arthritis pathogenesis.[7,8] A number of genetic associations in the form of alleles of genes such as TRAF1, STAT4, HLAII, CTLA4, PTPN22, TNFA-IP3, FCRL3, TNF-α, PADI4 and mRNAs have been described to confer an increased risk of development of rheumatoid arthritis in susceptible individuals.[9] In addition, single nucleotide polymorphisms (SNPs) has also been identified as an important risk factor playing a role in pathogenesis of rheumatoid arthritis.[10] In fact, as much as half of the genetic variations linked to susceptibility to rheumatoid arthritis are defined by more than 30 genes that have been identified so far.[11] Thus, highlighting the need to investigate the genes underplaying the susceptibility to rheumatoid arthritis in our population. Tumour necrosis factor alpha (TNFα) and its TNFα -308 polymorphism have increasingly been implicated in a number of autoimmune diseases including rheumatoid

arthritis.[12,13] Since TNFα -308 G is located in that region of TNFα gene which is responsible for secretion of TNFα, the association between rheumatoid arthritis and TNFα -308 G > can be explained.[14]

In view of increasing burden of rheumatoid arthritis and costs associated with the long-term morbidity caused by rheumatoid arthritis, a better understanding of its pathogenesis is warranted. This will enable knowledge of disease processes as well as identification of steps at which interventions can be aimed to lessen or eliminate the disease burden by development of better disease control or treatment strategies. This study was designed with an aim to study the association between susceptibility to rheumatoid arthritis and TNFα -308G/A promoter polymorphism in our population. A secondary objective was to determine the association between disease profile and TNFα -308 G/A promoter polymorphism.

MATERIAL AND METHODS

Following approval from ethics committee, this case-control study was conducted in the department of rheumatology PIMS Hospital Islamabad from 9th May to 9th August 2019 and enrolled patients with rheumatoid arthritis from among both the admitted patients and those attending the outpatient department. For the purpose of this study, patients belonging to either gender aged at least 18 years who were diagnosed as having rheumatoid arthritis according to the 2010 ACR/EULAR rheumatoid arthritis classification criteria were enrolled in this study. Age and gender matched healthy controls with no history of rheumatoid arthritis in first degree relatives were enrolled from general population. Patients younger than 18 years of age and those with features of additional or other auto-immune diseases on history or physical examination were excluded from the study. The study enrolled 150 patients diagnosed with rheumatoid arthritis using consecutive non-probability sampling technique and 150 age and gender matched controls. The sample size was calculated using the online sample size calculator at http://www.calculator.net/sample-size-calculator.html using the world health organization (WHO) protocol. After obtaining an informed consent, data was collected from patients using an interview-based questionnaire with a focus on patient demographics, socioeconomic status, clinical presentation, disease duration, seropositivity, laboratory investigations, medications and other health-related questions. Patients' blood samples were drawn under aseptic conditions before isolation of genomic DNA from blood samples using standard phenol-chloroform method. Next, DNA was quantified via spectrophotometry at 260/280 absorbance by using Nano drop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA quality was assessed using horizontal gel-electrophoresis on 1% agarose. Primer 3 v0.4 online tool available at http://bioinfo.ut.ee/primer3-0.4.0/primer3/ was used for designing forward and reverse primers for selected target single nucleotide polymorphism. PCR-RFLP method was used for genotyping of single nucleotide polymorphism. Data was entered into and analyzed using SPSS 16.2. Continuous variables were described as mean and standard deviation. Categorical variables were described as frequencies and percentages. The distribution of genotypes and alleles were compared between the groups and Chi-square ($\chi 2$) test was used to determine statistical significance. Risk associated with having a particular allele and / or genotype was assessed by calculating odds ratio (OR) with 95% confidence interval (95% CI). The correlation between demographic data (age, age of onset) and certain clinical features of RA patients with associated SNP were assessed using Chi-square ($\chi 2$) tests. Hardy-Weinberg equilibrium of genotype distribution was tested via Chi-square ($\chi 2$) goodness-of-fit test by comparing the observed and expected genotype frequencies among controls. A p value < 0.05 was considered as significant.

RESULTS

The mean±SD age of the patients was 44.83±12.37 years with a range of 20-74 years. Table-1 describes the descriptive statistics of the study population. There were 21 smokers in the study cohort and the mean duration of smoking among the study population was 8.72±4.59 years with a minimum duration of 2 years and a maximum duration of 18 years.

Table-2 shows frequency of different categorical variables in the study population. There were 35 (23.3%) males in the study. Majority (92%) of the study participants were married. Among smokers, majority of study participants (58%) had smoked cigarettes for less than 10 years. One hundred and fourteen (76%) study participants reported a monthly income of less than Rs. 20,000 per month indicating low socioeconomic status. Similarly, 48% of study participants were illiterate while graduates accounted for only 12% of study population. Interestingly, only 23% study participants believed that the disease had affected their job or daily progress significantly. Majority (60.7%) believed that the disease, i.e., rheumatoid arthritis had affected their job partially. The remainder (16%) reported no effect by the disease on their job or progress.

More than half of the study participants had not smoked a cigarette (65.3 %) while only 14% study participants were current smokers. All study participants were diagnosed cases of rheumatoid arthritis and 78.66% of study participants had a positive family history of rheumatoid arthritis. Interestingly, rheumatoid factor was found to be positive in a little more than half of the population (56%). No record for rheumatoid factor testing was available for 46 (30.7%) patients. This can be explained by the fact that patients at varying stage of disease were included in our study and many didn't have complete records available with them. Similarly, 123 (82%) patients did not have any record of anti-CCP antibody testing with them. It was positive in only 21 (14%). Almost 90% of patients did not have any co-morbid conditions, and among those, who did have co-morbid conditions, hypertension was the most common comorbidity in 17 (11.3%) patients followed by diabetes mellitus in 14 (9.3%) patients. Depression was diagnosed in 4 (2.7%) with rheumatoid arthritis and only 1 patient was currently on antidepressants (Table-2)

The genotypic analysis showed that GG genotype was the most common genotype found in 118 cases (78.66%) followed by GA (18.66%) and AA genotype (2.6%) p=0.0096 in both cases and controls. Overall, G allele was more common than A in both cases and controls pointing towards the preponderance of G genotype in our population. (p=0.003). However, the GA genotype and A allelotype was more common among cases with rheumatoid arthritis.

There was no difference among females between the frequency of GG, GA and AA genotypes when both cases and controls were compared (p 0.06). However, the G allelotype was more common in females among both cases and controls (p = 0.025). In males, however, the GA genotype was more expressed in cases compared to controls (p = 0.016). Similarly, expression of A allelotype and GA genotype was more in non-smokers compared to smokers ($p < 0.05$).

Table-1: Descriptive statistics of cases with rheumatoid arthritis

Variable	Minimum	Maximum	Mean	SD
Age (Yrs.)	20	74	44.83	12.370
Duration of smoking (years)	2	18	8.72	4.591
Duration of disease (years)	0.42	14.83	6.4911	4.53836
Das-28 Score	0.28	7.22	2.7467	1.31414
Hemoglobin (g/dl)	7	13	9.85	1.738
WBC count (10x3/ml)	4071	13890	8941.67	2428.510
Platelets (10x3/ml)	48	330	275.79	46.613
ESR (mm/hr.)	1	140	20.52	23.305

Table-2: Frequency of different categorical variables in study population

Variable	Frequency	Percent
Sex	**Frequency**	**Percent**
Male	35	23.3
Female	115	76.7
Total	**150**	**100**
Marital Status	**Frequency**	**Percent**
Married	138	92
Unmarried	12	8
Total	150	100.0
Duration of smoking	**Frequency**	**Percent**
Up to 10 years	87	58.00
More than 10 years	63	42.00
Total	150	100.0
Monthly income (Rupees)	**Frequency**	**Percent**
Less than 20 thousands / month	114	76.0
Between 20–50 thousands / month	27	18.0
More than 50 thousand	9	6.0
Total	**150**	**100.0**
Educational status	Frequency	Percent
Illiterate	72	48.0
Primary School Certificate	16	10.7
Secondary School Certificate	39	26.0
Higher Secondary School Certificate	5	3.3
Graduate	18	12.0
Total	**150**	**100.0**
How has the disease affected the job or progress	**Frequency**	**Percent**
Strongly affected	35	23.3
To some extent	91	60.7
Not at all	24	16.0
Total	**150**	**100.0**
Cousin Marriage	Frequency	Percent
Yes	80	53.3
No	70	46.7
Total	**150**	**100.0**
Tobacco Smoking Status	Frequency	Percent
Past Smoker	31	20.7
Never Smoked	98	65.3
Current Smoker	21	14.0
Total	**150**	**100.0**
Diagnosis	Frequency	Percent
Rheumatoid Arthritis	150	100.00
Total	**150**	**100.0**
Family History of Rheumatoid Arthritis	Frequency	Percent
Present	118	78.7
Absent	32	21.3
Total	**150**	**100.0**
Rheumatoid Factor	Frequency	Percent
Positive	84	56.0
Negative	20	13.3
Not Available	46	30.7
Total	**150**	**100.0**
Anti-CCP antibody	Frequency	Percent
Positive	21	14.0
Negative	6	4.0
Not available	123	82.0
Total	**150**	**100.0**
Comorbid conditions	Frequency	Percent
None	134	89.3
Diabetes Mellitus	14	9.3
Ischemic Heart Disease	1	.7
Epilepsy	1	.7
Total	150	100.0
Hypertension	Frequency	Percent
Present	17	11.3
Absent	133	88.7
Total	**150**	**100.0**
Depression	Frequency	Percent
Yes	4	2.7
Absent	146	97.3
Total	**150**	**100.0**
Current use of antidepressants	Frequency	Percent
No	149	99.3
Yes	1	0.7
Total	**150**	**100.0**

DISCUSSION

To our knowledge, this was the first ever study which focused on association of TNF-alpha -308 G/A promoter polymorphism with the spectrum of rheumatoid arthritis in our population. It turned out that G/A polymorphism is fairly common in our population and while both cases and controls in our study had a fair expression of G and A allelotypes, the GA genotypes and A allelotype was significantly more common among cases in our study.

The TNF-alpha -308 G/A promoter polymorphism has been known to be associated with a number of diseases such as schizophrenia[15], strong asthma risk[16], early onset sepsis in preterm infants[17] and a number of other conditions.[18–20] The TNF-alpha -308 G/A promoter polymorphism has also been known to play a role in rheumatoid arthritis.[10,13,]

A case control study from Bulgaria studied TNF-alpha -308 G/A promoter polymorphism in 177 controls, 108 cases of rheumatoid arthritis, and 58 cases of ankylosing spondylitis.[21] Interestingly, the study concluded that TNF-alpha -308 G/A promoter polymorphism had no significant effect in development of rheumatoid arthritis when compared with controls as well as patients with ankylosing spondylitis. These findings are echoed by another study, from Argentina this time, which studied the TNF-alpha -308 G/A promoter polymorphism in 223 patients with rheumatoid arthritis and 111 age and gender matched controls. The study found no significant differences in the TNF-alpha -308 G/A promoter polymorphism between the cases and controls.[22] On the other hand, we observed a statistically very significant difference between cases and controls as far as TNF-alpha -308 G/A promoter polymorphism was concerned. The proportion of A allele in our study was more in cases compared to controls and although, no statistically significant association was found between the duration of disease and the allele types in our study, it has been observed that patients with rheumatoid arthritis who have the G allele respond better to anti-TNF alpha treatment for rheumatoid arthritis.[23]

A similar study from Egypt reported that presence of GG genotype was associated with susceptibility to rheumatoid arthritis and presence of A allelotype was associated with presence of bony erosions in their study population.[24] Additionally, they observed no associations between the G/A genotypes / allelotypes and a number of risk factors such as duration of disease, disease remission, age of patients, clinical disease manifestations, bony deformities and presence of rheumatoid factor positivity.[24] Our results support the above findings.

To sum up, genes underplay an important role in the autoimmunity that forms the basis of rheumatoid arthritis and while the TNF-alpha -308 G/A promoter polymorphism has been identified as an important etiopathogenic factor in the disease progression, conflicting reports are available in the literature. In view of these conflicting reports, it is recommended that future studies with larger sample size be conducted to study the magnitude of TNF-alpha -308 G/A promoter polymorphism in the natural history of rheumatoid arthritis.

Our study was limited by the fact that that it was a single centre-based study with a relatively small sample size. Most of the patients in the study were females. Therefore, the association of gender with TNF-alpha -308 G/A promoter polymorphism could not be properly highlighted.

AUTHORS' CONTRIBUTION

SK: Literature search, Data collection, study design, manuscript writing. AZ, SB, MSK: Data collection, analysis, literature search. UR, SZ: Data interpretation. WA: Expert opinion.

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Submitted: September 15, 2019	*Revised: November 3, 2019*	*Accepted: February 23, 2020*

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