

RESEARCH PAPER

Association of Interleukin-10 Gene Polymorphism with Susceptibility and Severity of Axial Spondyloarthritis

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Abstract

Background: This study aimed to determine the association of Interleukin-10 (IL-10) gene polymorphisms with the susceptibility and severity of axial spondyloarthritis (axSpA) that may guide to have an idea about the genetic basis of the disease and its association with severity.

Objective: Aim of the study was to demonstrate the IL-10 gene polymorphisms to determine their association with susceptibility and severity of axSpA.

Methods: According to Assessment of Spondyloarthritis International Society (ASAS) classification criteria total 38 patients with axSpA (clinically diagnosed by an expert Rheumatologist, attending in outpatient department (OPD) of Rheumatology, Bangabandhu Sheikh Mujib Medical University, BSMMU) and 38 healthy controls (resident doctors, laboratory staffs of BSMMU and general people) after fulfilling the inclusion criteria were enrolled in this study. Blood samples were collected after taking informed written consent and data were collected in a predesigned data collection sheet. The IL-10 gene polymorphisms IL-10 (819T/C), IL-10 (1082A/G) and IL-10 (592C/A) were detected by Polymerase chain reaction- Restriction fragment length polymorphism (PCR- RFLP) method at Department of Microbiology and Immunology, BSMMU, Dhaka.

Results: According to BASDAI score, 14 patients were in inactive disease group and 24 patients were in active disease group. The homozygous CC genotype and C allele of IL-10 (-819T/C) were found significantly higher in patients than control group ($p=0.022$ and $p=0.045$ respectively). The heterozygous GA genotype of IL-10 (-1082A/G) gene has significant association ($p=0.001$) with axSpA. It indicates that the homozygous CC genotype and C allele of IL-10 (819T/C) and heterozygous GA genotype of IL-10 (1082A/G) have an association with the susceptibility of axSpA.

Conclusion: CC genotype and C allele of IL-10 (-819T/C) and GA genotype of IL-10 (1082A/G) are associated with axSpA susceptibility. But no association of these genotypes were found with disease severity.

Keywords: IL-10, PCR-RFLP, BASDAI

Introduction

Spondyloarthritis (SpA) includes peripheral SpA and axial SpA; the latter is distinguished by a predominance of spine or sacroiliac joint involvement. Ankylosing spondylitis (AS), the radiographic type of axial SpA, and nonradiographic axial SpA (nr-axSpA) are both classified as axial SpA (axSpA). Both conditions can be seen as two distinct stages

of the same¹. The majority of persons with this condition are between the ages of 20 and 60 years, which places a significant socioeconomic burden on their families.² The estimated prevalence of AS in the world's population ranges from 0.7% to 3.2%.³ Prevalence in Bangladesh is 1.2% (0.7-1.8).⁴ Although the dominant gene linked to AS, human leucocyte antigen (HLA)-B27, only 1%–5% of patients are HLA-B27 positive.⁵ In addition, the distribution of HLA-B27 and its subtypes around the world differs significantly. According to reports, the prevalence of HLA-B27 positive in the Chinese and Korean populations is 4–8% and 2.3–7%, respectively. This is lower than the frequency among

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Caucasians but higher than that of the Japanese population 1%.⁶ The relationship between genetic factors and non-MHC variations and illness risk has been studied. A genome-wide association study (GWAS) has so far identified at least 36 genetic variations in non-MHC areas as being linked to AS.⁷ Numerous studies have demonstrated the value of cytokine gene polymorphism as an early prognostic marker and for earlier selection of the most effective medication.⁸ Studies have been done on cytokines that promote inflammation. However, research on anti-inflammatory cytokines is scarce. Numerous cytokines and immune response regulators' expression levels are also genetically influenced. Therefore, it is highly probable that genetic variations might affect the pattern of cytokine release in AS.⁵ IL-10 has been demonstrated to have anti-inflammatory characteristics and a role in avoiding inflammatory and autoimmune diseases.⁹ It is largely produced by monocytes and lymphocytes. It controls the balance of Th1 vs. Th2 cytokines, which is a key factor in controlling the balance between immunity and autoimmune disease, by down-regulating the expression of Th1 cytokines. The promoter of IL-10, which is extremely polymorphic, also affects the production of IL-10.⁹ The IL-10 gene on chromosome 1q31-32 encodes IL-10, an immunomodulatory cytokine that has five exons and four introns. The production of IL-10 is known to be regulated by polymorphisms at locations 1082 A>G, 819 T>C, and 592 A>C in the 5' flanking region of the IL-10 gene.¹⁰

The clinical Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is frequently used to assess disease activity in AS patients by using a questionnaire.¹¹ The tiredness, joint pain/swelling, localized soreness, fatigue, and morning stiffness are all included in the BASDAI. The range of possible BASDAI scores is 0 to 10. A score less than 4 denotes inactive disease, whereas a score more than 4 is indicative of an active illness.⁸

A Chinese study as well as a recent investigation of a mixed ethnic group revealed that the genotypes A/G+G/G and G alleles of IL-10-1082 were substantially linked to the risk of AS. They discovered that this connection was unrelated to age or the presence of HLA-B27, which strengthens the case for an association between the IL-10 gene and AS.¹⁰

Despite the difference in demographic, a different study that included a Chinese population also discovered a relationship between the same polymorphism and AS, as well as with the IL-10 -1082AG and IL-10 -819CC genotypes.⁵ Other research produced a variety of outcomes, which may be related to racial and ethnic

variances.⁵ The purpose of this study is to investigate the potential relationship between three single nucleotide promoter polymorphisms (SNPs) at IL-10 (-1082A/G, -592C/A, and -819T/C) and the susceptibility and severity of axial spondyloarthritis.

Materials and Methods

In this case control study, a total number of 38 axSpA patients attending at OPD of Rheumatology, BSMMU were selected as cases and 38 healthy controls were selected from resident doctors and laboratory staffs and general people of same geographical area.

Patients were selected according to the ASAS criteria for diagnosis of axSpA after taking informed written consent. Persons having no diagnosed autoimmune and/ or rheumatological diseases, those were unrelated to patient group and belonging to the same ethnic group as the patients were selected as controls.

Pregnancy, alcohol abuse, acute infection and uncontrolled diabetes were the exclusion criteria for cases.

Person having family history of axSpA and other rheumatological disorder, pregnancy and acute infection were the exclusion criteria for controls.

Peripheral venous blood samples were collected and genomic DNA was extracted according to manufacturer's instructions (Genomic DNA extraction Kit, Anatonnia Geneworks, Bosphore, Turkey). Extracted DNA were stored at -20°C until further use.

IL-10 Genotyping: IL-10 SNPs at the positions 819 T/C, 1082 A/G and 592 C/A were amplified by PCR and detected by RFLP technique. The PCR reaction was performed using a 20 µl reaction mixture containing 15 µl of master mix (Takara Bio Inc.), 3 µl of nuclease free water, 1 µl of each forward and reverse primers. Then 5 µl of extracted DNA was added.

For position 819C/T, forward and reverse primers are 5AGTAAGGGACCTCCTATCCAGCC3 and 5CTCAAAGTT CCAAGCAGCC3. For position 1082 G/A, forward and reverse primers are 5CACACAAAT CCAAGACAACACTACTAAGGCTTCTTTCTGA3 and 5ATAGTGAGCAAACCTGAGGCACAGAG3. For position 592 C/A, forward and reverse primers are 5GGTGAGCACTACCTGACTAGC3 and 5CCTAGG TCACAGTGACGTGG3.

Amplification were carried out in proflex PCR system (Applied Biosystems, Thermo fisher scientific, USA) as follows: For 819C/T, initial denaturation at 94°C for 3 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 35 seconds and final extension at 72°C for 5 minutes. For 1082 G/A, initial denaturation

at 94°C for 10 minutes, 40 cycles of 94°C for 30 seconds, 60°C for 60 seconds, 72°C for 10 seconds and final extension at 72°C for 1 minute. For 592 C/A, initial denaturation at 94°C for 3 minutes, 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 5 minutes.

RFLP was performed by digesting with restriction enzyme (MslI for 819C/T, AclI for 1082 G/A and RsaI for 592 C/A) according to manufacturers guidelines (New England Biolabs, Ipswich, MA, USA) at 37°C overnight followed by electrophoresis on 2% agarose gel stained with ethidium bromide.

For 819T/C T allele, PCR product were uncut 520bp. For C allele, PCR product were cleaved into two fragments with 279 and 241bp. For 1082 G/A G allele, PCR product were uncut 331bp. For A allele, were cleaved into 289bp and 42bp. For 592 C/A C allele, PCR product were uncut 412bp. For A allele, cleaved into two 176bp and 236bp. (Figure- 1)

All the data were rechecked, coded, entered in a data base, and analyzed using SPSS software (Version-22). The categorical variables were expressed as numbers (n) and percentages (%), while continuous variables were expressed as Mean ± Standard Deviation. Comparisons of categorical data were performed using chi-square test. The strength of association was assessed using odds ratio (OR) and

95% confidence interval (95% CI). For all statistical analysis p-value less than 0.05 was considered statistically significant.

Results

Clinico-demographic characteristics of 38 axSpA patients shows the age range is 21-49 years. Among 38 cases 24 are male, 27 have peripheral arthritis, 26 have enthesitis, 25 have dactylitis, 6 have uveitis, 8 have psoriasis, 4 have IBD and 25 have positive family history. Mean age at onset is 31.5±7.92 years and disease duration is 3.18±1.93 years. Mean CRP level is 26.9±17.5 mg/L. On X-ray all patients had sacroiliitis and 34 patients are HLA-B27 positive.

The genotype distributions of IL-10 single nucleotide polymorphisms are shown in table- I. IL-10 (-819T/C) showed that, the homozygous mutant CC genotype was found in 9 patients and in 2 controls ($p=0.022$). C allele was found in 35 cases and in 23 controls ($p=0.045$).

The genotype distribution of IL-10 (-1082 G/A) showed, heterozygous GA was found in 29 patients and in 12 controls ($p=0.001$). Homozygous AA was found in 8 patients and 20 healthy controls ($p=0.004$).

IL-10 single nucleotide polymorphisms by PCR-RFLP are shown in figure- 1.

Table I: Comparison of allele and genotype frequencies of IL-10 (819T/C), IL-10 (592 C/A) and IL-10 (1082A/G) in axSpA patients and controls (n=76)

Genotype and allele	Case (n=38)	Control (n=38)	OR (95% CI)	p-value
IL-10 (819 T/C)				
CC	9	2	5.58 (1.12-27.90)	0.022*
TT	12	17	0.57 (0.22-1.45)	0.238
CT	17	19	0.81 (0.33-1.99)	0.646
C allele	35	23	1.97 (1.01-3.82)	0.045*
T allele	41	53		
IL-10 (592 C/A)				
CC	18	11	2.21 (0.86-5.69)	0.098
CA	20	27		
A allele	20	27	0.65 (0.32-1.29)	0.219
C allele	56	49		
IL-10 1082 A/G				
AA	8	20	0.24 (0.09-0.658)	0.004*
GG	4	6	0.63 (0.16-2.43)	0.497
GA	26	12	4.69 (1.78-12.35)	0.001*
A allele	42	52	0.57 (0.29-1.11)	0.095
G allele	34	24		

Note: OD= Odds ratio, CI= Confidence interval, p-value reached from Chi- square test, *p-value <0.05 indicates significant.

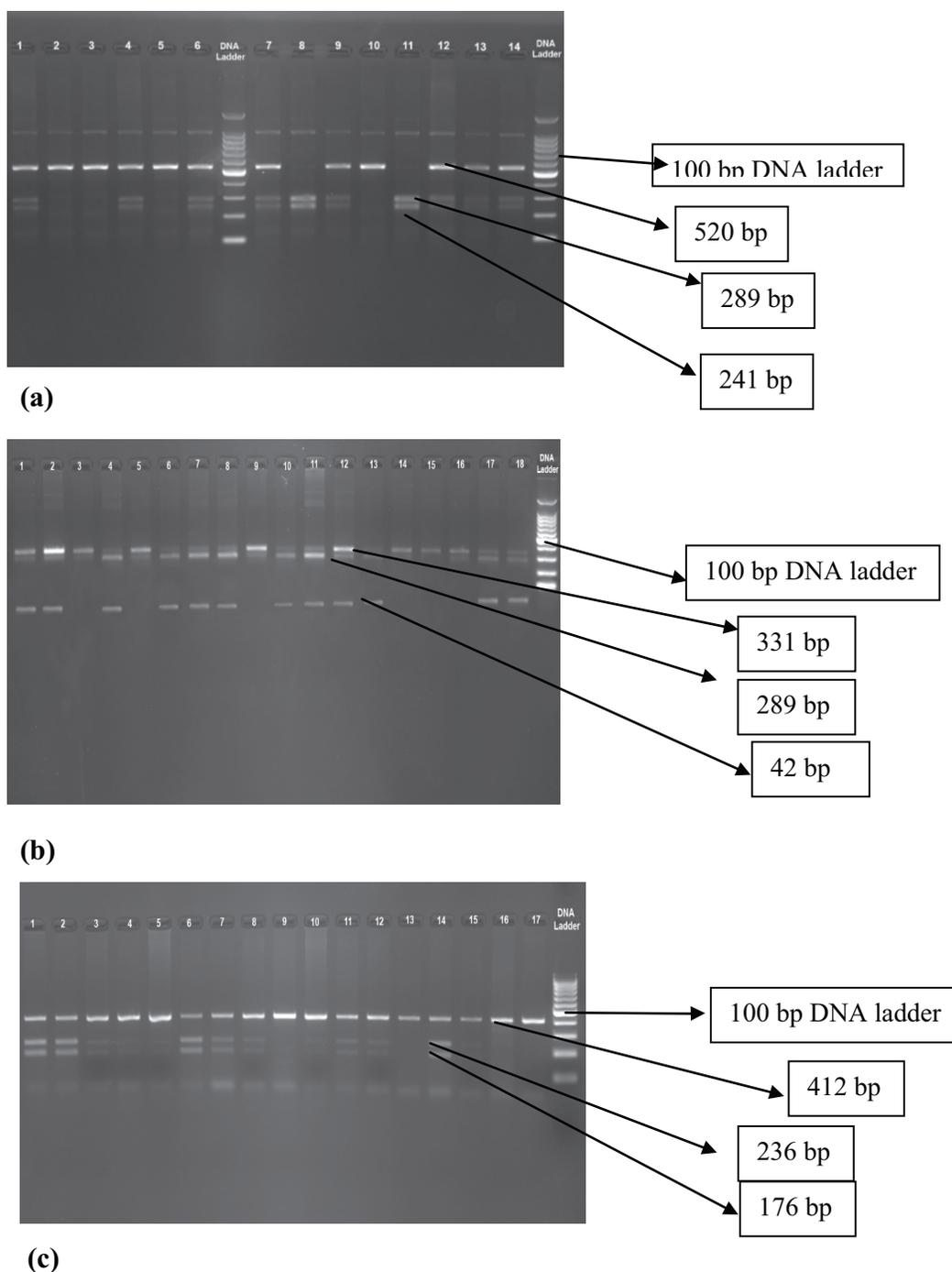


Figure 1: PCR-RFLP analysis of (a) IL-10 (-819T/C) (b) IL-10 (-1082 G/A) and (c) IL-10 (-592C/A) polymorphism by agarose gel electrophoresis.

The disease activity were categorized into 2 groups according to BASDAI score. This includes inactive disease group (score <4) and active group (score >4). Among ax-SpA patients,

14 patients were in inactive group and 24 patients were in active group.

No associations of IL-10 gene polymorphisms with degree of severity of axSpA patients were found according to BASDAI score.

Table II: Association of IL-10 gene polymorphisms with the degree of severity of axSpA patients according to BASDAI score (n=38)

	BASDAI	Genotypes				Allele		
		CC	TT	CT	p-value	C	T	p-value
IL-10 (819 T/C)	<4	3	2	9	0.133	15	13	0.315
	>4	6	10	8		20	28	
IL-10 (1082 A/G)	<4	AA	GG	GA	0.873	A	G	0.801
	>4	3	1	10		16	12	
IL-10 (592 C/A)	<4	CC	CA	AA	0.804	C	A	0.842
	>4	7	7	0		21	7	
		11	13	0		35	13	

Note: BASDAI= Bath Ankylosing Spondylitis Disease Activity Index, p-value reached from Chi- square test Besides, no associations were found among IL- 10 819T/C, IL- 10 1082 G/A and IL-10 592 C/A single nucleotide polymorphisms with IL-10 levels.

Discussion

This study looked into whether axial spondyloarthritis susceptibility and severity were correlated with single nucleotide polymorphisms (SNPs) of IL-10. Multiple genome-wide association studies (GWAS) have established a connection between non-HLA genes and axial spondyloarthritis. In this investigation, there was a substantial difference in the genotypic distribution of the IL-10 (1082A/G) gene polymorphism between patients and healthy controls. In patients, the heterozygous mutant GA genotype was more common than in the healthy control group (29 vs 12) ($p=0.001$). In comparison to healthy controls, the homozygous wild type AA was found to be considerably lower in patients (8 vs 20) ($p=0.004$). The results of this investigation are consistent with those of other studies conducted in Brazil and China, which discovered that patients had considerably greater heterozygous mutant GA genotypes than control groups (p values of 0.001 and 0.034, respectively).^{5,10} This study also contrasted axial spondyloarthritis patients with a healthy control group with IL-10 (-819T/C) gene polymorphisms. The heterozygous mutant genotype CC was found in 9 cases and in 2 healthy controls ($p=0.022$), according to the genotype distribution between patients and healthy control groups. In the case group, the C allele frequency was greater than in the control group (35 vs 23) ($p=0.045$). Similar to this, a meta study carried out in China revealed a link between AS vulnerability and the CC genotype and C allele.¹⁴ In contrast, Lv et al. (2011) demonstrated that AS susceptibility is

correlated with both the CC genotype and the C allele. Between controls and patients with axSpA, there was no discernible variation in the genotype and allele frequencies of IL-10 (592C/A) in the current investigation. Chinese meta study likewise discovered no connection between AS susceptibility and the genotype of IL-10 (592C/A).¹⁴ It was discovered in this study that the IL-10 (1082A/G), IL-10 (819T/C), and IL-10 (592C/A) genotypes do not significantly correlate with serum levels of IL-10 in the patient group. When compared to the -1082AA genotype, Lv et al. (2011) discovered that the IL-10 (1082AG) genotype was related with greater IL-10 levels ($p=0.001$). When compared to the -819TT genotype, the -819CC genotype was linked to greater IL-10 levels ($p=0.041$). However, unlike this study, Braga et al. (2021) observed no significant correlation between the IL-10 (1082A/G) genotype and IL-10 level, indicating that other SNPs may have an impact on the cytokine's production.¹⁰ Other than these, no significant correlation between the severity of axSpA and IL-10 gene polymorphisms was discovered in this investigation. Additionally, Braga et al. (2021) discovered no connection between the IL-10 gene variation and the severity of the illness. Therefore, the purpose of this study was to explore any potential associations between axial spondyloarthritis susceptibility and severity with IL-10 gene polymorphism.

Conclusion

GA genotype of IL-10 (-1082A/G) is associated with the susceptibility to axSpA. CC genotype and C allele of IL-10 (-819T/C) are also play role in susceptibility

to axSpA. There is no association of these genotypes with disease severity.

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