



A STUDY OF INTERLEUKIN-15 (IL 15) GENE POLYMORPHISMS IN PAKISTANI PATIENTS WITH RHEUMATOID ARTHRITIS (RA)

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Abstract-

Background- Aim of the present study was to explore the association of two single nucleotide polymorphisms (SNPs), including rs4956403 (SNP-1) and rs3806798 (SNP-2) located in the 5' UTR region of interleukin 15 gene in rheumatoid arthritis of Pakistani patients. Interleukin-15 is one of the key cytokines in the pathogenesis of rheumatoid arthritis. The genetic polymorphisms located in the regulatory regions of interleukin-15 gene might play a role in rheumatoid arthritis.

Methodology- Fresh blood samples were collected from different hospitals. Allele specific PCR was employed for the analysis of these polymorphisms. Results were analyzed statistically for any significant association of these polymorphisms with rheumatoid arthritis.

Results- The results of our study showed that SNP-1 and SNP-2 located in interleukin-15 gene are not associated with rheumatoid arthritis in our patients.

Conclusion- Any other Single nucleotide polymorphisms (SNPs) located in the regulatory and untranslated regions of IL15 gene may have association with rheumatoid arthritis, for which large scale data is required. Other molecules implicated in the inflammatory pathway of Rheumatoid Arthritis needs to be investigated.

Keywords- Interleukin 15, Rheumatoid arthritis, Polymorphism, SNP, Pakistan, DNA, Primers, Genotype

Running Title- IL 15 Gene Polymorphism in RA.

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Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease that targets synovial membranes, bones and joints. Worldwide, its prevalence is about 0.3-to 1.2% [1]. It is a multi-factorial disease and a number of factors contribute to the development and pathogenesis of the disease. Contribution of genetic factors towards RA ranges from 50-60% [2,3]. Human leukocyte Antigen (HLA) locus is the most important genetic risk factor in RA [4,5]. Major environmental factors that contribute to increased risk of RA include smoking, diet and hormones. Smoking, however, is usually considered the most important risk factor. Smoking can lead to 1.3-2.4 times increased risk of RA [6]. Genome Wide Association Studies (GWAS) have emphasized on the association of single nucleotide polymorphisms (SNPs) with RA. SNPs are particularly useful as every individual is uniquely characterized by a set of at least 3 million common SNPs that are distributed across the genome. SNPs, in turn, can determine the response of an individual towards differ-

ent environmental factors.

Cytokines are important component of the immune system. These cytokines integrate the immune regulatory pathways that ultimately lead to different autoimmune diseases including RA. T-helper cell (T_H cell) cytokines are the most important of all the cytokines that might play a role in RA [7]. Interleukin-15 (IL 15) is an important T cell cytokine that shares structural similarity to interleukin-2 (IL-2). The receptor for these two cytokines is complex, composed of IL-12/IL 15 receptor beta chains (Cluster of differentiation-122, CD-122) and a common gamma chain (Cluster of Differentiation-132, CD-132). It is primarily secreted by mononuclear phagocytes. It regulates T and natural killer cell activation and proliferation.

The levels of IL 15 have been observed to be increased in RA as compared to other rheumatic diseases [8]. It is secreted by fibroblasts and macrophages in the inflamed synovial membrane during RA [9]. Increased levels of this cytokine may lead to the recruitment

and activation of synovial T-cells [10]. It leads to the differentiation of the osteoclast progenitors which is the key step that leads towards the bone deterioration [11].

The expression of IL 15 is regulated at both transcriptional and post-transcriptional levels. Genetic polymorphisms in the genes of the cytokines can lead to the variations in the levels of these cytokines in the body [12]. Thus polymorphisms located in the regulatory regions of IL15 gene might play a role in pathogenesis of immune system diseases.

The above mentioned findings and observations encouraged us to perform a case-control association study in Pakistani patients to examine the role of IL 15 gene polymorphisms in the susceptibility to RA. To the best of our knowledge this is the first case control study in context to Pakistani RA patients.

Materials and Methods

The work designed was a case control study. As a whole, 100 cases and 100 controls were included in the study. The patients included in the case group were clinically diagnosed by rheumatologist and the American Criteria of Rheumatology (ACR-2011) criterion was used. Written informed consent was obtained from the patient to participate in the study. The ratio of females and males included in case group was 80% and 20% respectively. The average age of the patients included in the study was 46.38 ± 10.67 years. The

ratio of females and males in the control group was 77% and 23% respectively. The average age of the healthy individuals was 32.68 ± 5.79 years. Blood samples were collected from the affected and normal individuals. Genomic DNA was extracted from blood samples by phenol-chloroform method and was used for PCR amplification. SNPs selected for the association study are located in 5' UTR region of IL 15 gene at position -831 (rs4956403 / SNP-1) and -475 (rs3806798 / SNP-2). Primers were manually designed for allele specific PCR [Table-1].

Allele specific PCR was carried out to check the presence of polymorphisms in the study population. PCR conditions included a template denaturation at 95°C for 5 minutes followed by 35 cycles of PCR amplification. Each PCR cycle further consisted of 3 steps: 1 minute at 95°C for the denaturation of template DNA strands into single strands, 45 seconds at 55°C to allow the annealing of the primers to their respective target sites on DNA and 1 minute and 20 seconds for extension of the complementary DNA strand from the annealed primers. These 35 cycles were followed by synthesis of any unextended strands by Taq polymerase for 10 minutes at 72°C. PCR products were then analyzed by 2% agarose gel. Data was statistically analyzed by using Statistical Practices for Social Sciences (SPSS) and Fischer exact test was applied for association analysis of the polymorphisms in the study population Graphpad PRISM.

Table 1- Primers designed for SNP-1 and SNP-2 located in the regulatory region of interleukin-15 (IL 15) gene. The mutated nucleotide has been indicated in red color

SNP	Database identification	Gene Location	Change	Forward Primers	Reverse Primers	Product size (base pairs)
SNP-1 rs4956403		5' UTR	C→T	5'1GGAAGACATCATTTTCTCTGAACC13' 5'1GGAAGACATCATTTTCTCTGAAC13'	5'1ACAGGCTGCAAGAAGGAACTTT13'	461
SNP-2 rs3806798		5' UTR	T→A	5'1AACATTTCCCTAGTTGGACT13' 5'1AACATTTCCCTAGTTGGACA13'	5'1AAAGAGAAAGAGCCGGGAG13'	470

Result

The genotype frequencies of the screened polymorphisms were not found to be significantly different from those predicted by Hardy-Weinberg equilibrium (HWE) in healthy subjects. All the possible combinations of alleles were observed in the study subjects for both of the SNPs [Fig-1]. The observed genotype frequencies have been illustrated in [Table-2]. The frequency of all the possible genotypes did not showed any significant difference in the patients and the healthy individuals [Table-2].

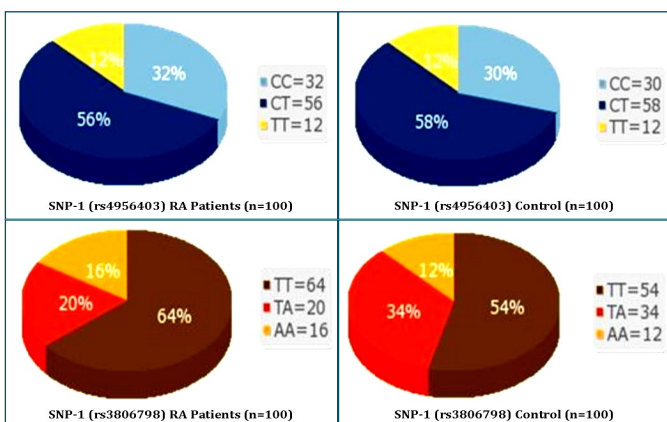


Fig. 1- Distribution of the study subjects across different genotypes for the studied polymorphisms.

Correlation of respective SNP's with clinic-pathological findings and age has been shown in [Table-3]. The association analysis showed

that the genotypes of the individual, with respect to respective SNPs, were not linked to any of the disease attribute or gender of the individual [Table-3].

Discussion

The levels of IL 15 are known to increase significantly in RA patients [8]. The expression of this cytokine is known to be regulated at both pre-transcriptional and post-transcriptional levels. SNPs located in the regulatory regions of this cytokine can therefore play a significant contribution towards pre-disposition towards RA. However, the results of our study did not confirm any considerable association of these two polymorphisms with RA.

The SNPs located in IL15 gene might affect the characteristic clinical features of the disease as these polymorphisms can lead to altered levels of this cytokine. These altered levels can in turn determine the extent of damage caused by the effector cells in the inflamed synovium and rheumatoid joints. These polymorphisms can thus determine the clinical presentation of RA. The association of these polymorphisms was therefore studied in relation to different characteristic features of RA. These characteristic features included age of onset of the disease, duration of symptoms, presence of migratory arthritis, the duration of morning stiffness and the severity of the disease. The association analysis of the observed genotypes and the occurrence and extent of these clinical features indicated that there was no significant association between these clinical features of the disease and IL15 gene polymorphisms being studied. This indicates that the actual genetic determinants of the clinical features of RA are located either at other positions in IL15 gene

or they might be located in the genes of other candidate genes for RA.

RA is more common among females. The polymorphisms that affect the regulation of key regulatory molecules involved in the pathogenesis of RA might contribute towards RA in a sex-dependent manner. The association of rs4956403 and rs3806798 was therefore analyzed in RA patients in relation to the sex of the patient, in our study. The results of the statistical analysis showed there was no significant difference between RA affected females and males in relation to the occurrence of these polymorphisms.

The results of our study indicated no significant association of any

of the studied IL15 gene polymorphisms in RA patients of Pakistani origin. These results are consistent with a previous association study conducted in Spanish population, where 13 different SNPs were analyzed in association to RA. These 13 SNPs included six SNPs located in 5' UTR region, three SNPs located in the intronic region and four located in 3'UTR region. The resulting data indicated no significant difference in the distribution of the observed genotypes in RA patients and healthy individuals. rs4956403 and rs3806798 located in the 5' UTR region of IL15 gene, were also analyzed in that study and were not found to be significantly associated with RA [13].

Table 2- Genotype and allele frequencies of SNP-1 and SNP-2 in RA patients and controls

SNP	dbSNP number	Alleles		Patients (n=100)			Controls (n=100)			MAF*	MAF*	p-value
		a	b	Homozygous (aa)	Heterozygous (a/b)	Homozygous (bb)	Homozygous (aa)	Heterozygous (a/b)	Homozygous (bb)	Patients	Controls	
SNP-1	rs4956403	C	T	32	56	12	30	58	12	40	41	0.3
SNP-2	rs3806798	T	A	64	20	16	54	34	12	26	29	1

*MAF=Minor Allele Frequency

Table 3- The association of SNP-1 and SNP-2 with different clinical features of rheumatoid arthritis (RA) and sex of the patient

SNP	db SNP number	p-value					
		Age of onset	Duration of symptoms	Duration of morning stiffness	Migratory arthritis	Disease severity	Sex
SNP-1	rs4956403	0.1	1.7	0.7	0.4	0.8	0.4
SNP-2	rs3806798	0.5	0.4	0.3	0.1	0.4	0.3

Conclusions

In summary, the results of our study showed that SNP1 and SNP2 in IL 15 gene are not associated with RA in studied Pakistani patients. However, other molecules implicated in the inflammatory pathway of RA, needs to be investigated for association with RA in disease pathogenesis. Other polymorphisms of IL 15 located in the regulatory and untranslated regions may have significant association towards RA, for analysis of which large scale data is required.

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Conflict of Interest

This study has no conflict of interests. All authors have seen the original manuscript and they have approved it.

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