ROLE OF IL10 -592C/A GENE POLYMORPHISM WITH ASTHMA IN A NORTH INDIAN POPULATION

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ABSTRACT: Changes in the climate and air quality increase the incidence and prevalence of allergic diseasesincluding asthma. Deforestation as well as air pollution lead to global warming that has the potency to regulate the initiation, duration and intensity of the pollen season which exacerbate the disease. The risk of disease also occurs due to interactions between more than 100 susceptible genes and multiple environmental factors. Furthermore, single nucleotide polymorphism influences the production as well as expression of particular gene and it has been found that low level of IL10 influence the asthma severity. Therefore, the present study was conductedto examine the role of IL10-592C/A polymorphism in asthma with a total of 964 subjects, including 483 healthy controls and 481 asthma patients. DNA samples were extracted from blood and genotyping of IL10 -592C/A was done usingamplification refractory mutation system- polymerase chain reaction (ARMS-PCR) method. Statistical analysis revealed a non-significant association towards asthma in both the allelic as well as genotypic frequencies with OR=1.00 and p=1.00. In conclusion, this study found that IL10 -592C/A polymorphism is not associated with asthma in a North Indian population.

Keywords*: Asthma; IL10 polymorphism; North India; pollution; total IgE*

I. INTRODUCTION

Asthma is a respiratory condition that results due to the complex interplay of genetic as well as environmental factors and occur at critical times in an individual developmental stages [1]. Although, the proportional contribution of each factor in disease susceptibility is still unknown, disease expression is associated with the several major and minor genes interaction and further modulation by the various environmental factors.According tothe 'World Allergy Organization' (WAO),changes in climate and air quality increase the incidence and prevalence of allergic diseases that have a measurable impact on the asthma patients. Deforestation leads to the change in climate that eventually influences the botanical allergen production and their complex interactions which result in intense human susceptibility to various immune disorders. Deforestation as well as air pollution leads to global warming that has the potency to regulate the initiation, duration and intensity of the pollen season that exacerbate asthma. Other factors such as high density population, poor sanitation, dust, *etc.* also contribute to the disease severity [2].

Interleukin 10 (IL10) is a pleiotropic, anti-inflammatory cytokine having pathophysiologic mechanism of auto-immune and inflammatory disease [3]. This process is carried out through down-regulation of pro-inflammatory cytokines [4]. IL10 is produced by almost all leukocytes including $CD4^+$ and $CD8^+$ T lymphocytes, mast cells and activated monocytes [5]. The generation of allergen-specific Th2 cells provide the initial event responsible for the development of allergic diseases and decrease expression of IL10 produced by Th2 cells have been found in the serum of allergic patients [6,7]. From mouse model, it has been also observed that IL10 inhibits eosinophil survival as well as IL4 induced IgE synthesis [8]. The human IL10 is present on chromosome 1q31-32 that is investigated as a genomic region linked to asthma and related phenotypes [9]. The IL10 promoter region is highly polymorphic and has three important SNPs lie in the putative transcription factor binding site which increases the expression of IL10in the presence of mutant allele. These SNPs are -1082A/G (rs1800896), -819C/T (rs1800871), and -592C/A (rs1800872) [10].

In thepresent study, the -592C/A polymorphism has been studied as the mutant A allele of -592C/A polymorphism decreases the level of IL10 production while wild C allele increases the production of IgE that result in a more severe bronchial asthma [11].Also,the role of IL10 -592C/A polymorphism in correlation with several asthma related parameters such as gender, age, disease duration, atopic status, smoking status, family history, IgE level, pulmonary function *etc.* has been assessed in a North Indian population.

II. MATERIALS AND METHODS

A. ETHICAL CLEARANCE

Ethical clearance was granted by the Ethics Committee, PGIMER, Chandigarh, India, for conducting this research work and it is strictly in accordance with the "Ethical Guidelines for Biomedical Research on Human Participants (2006), Indian Council of Medical Research and Ministry of Health, Govt. of India and in compliance with the Declaration of Helsinki (1964). After doctor's diagnosis each patient were

provided with written information about the study and a due consent was taken from each patient prior to inducting him/her in the study.

B. INCLUSION/ EXCLUSION CRITERIA

Patients from different states of North India including Chandigarh, Punjab, Haryana, Himachal Pradesh, Uttaranchal, Jammu and Kashmir, Rajasthan, Uttar Pradesh, and New Delhi were recruited. A total of 481 patients (191 males and 290 females) enrolled as cases visiting OPD (Out Patient Department), Pulmonary Medicine at PGIMER, Chandigarh, and 483 (189 males and 294 females) age-matched, healthy individuals, without any symptoms of atopic, pulmonary disease, any other co-morbid disease or smoking habits were recruited as controls. Apart from these, any other co-morbid illness such as diabetes mellitus, hypertension or pregnant females were not recruited as cases in this study.

C. LUNG FUNCTION TEST

Spirometry tests were performed according to Association of Respiratory Technician and Physiologists (ARTP) guidelines [12] for generating pneumotachographs, helpful in assessing conditions such as asthma, COPD *etc.* usingSpiro 233 (PK Morgan, Rainham, Kent, UK).Out of 481 asthma patients, spirometry was done in 377 asthmatics which are further categorized according to their severity.

D. TOTAL IgE MEASUREMENT

Total IgE was measured using ImmunoCAPS with device Phadia 100 IDM version 5.43 (Thermo Fisher Scientific Inc., Waltham, MA, USA) in serum samples of both control and asthma patients to screen allergy.

Skin Prick Test (SPT) and serum specific IgE against *Aspergillus fumigatus* were also done in some patients to distinguish asthma and ABPA. Only negative SPT patients and serum specific IgE<0.35 KUA/L were recruited in the study (Table 1).

E. BLOOD SAMPLE COLLECTION

Approximately 5ml blood was collected from each patient as well as control subjects in EDTA coated vials and stored at -80ºC until extraction of genomic DNA was done.DNA was isolated from frozen whole blood samples using SSC Buffer method [13] and checked on 0.8% agarose gel before storage at -20ºC for further use.

F. GENOTYPING

The amplification of the IL10 -592C/A polymorphism were done using ARMS- PCR and specific primers were [14]

Control (HGH) F 5'-CCTTCCAACCATTCCCTTA- 3'
Control (HGH) R 5'-TCACGGATTTCTGTTGTGTTTC- 3'
Common 5'-TAACTTAGGCAGTCACCTTAGG- 3'
IL10 -592 (A) 5'-ACATCCTGTGACCCCGCCTGTA- 3'
IL10 -592 (C) 5'- ACATCCTGTGACCCCGCCTGTC-3'

The PCR was carried out in a thermal cycler, in a total volume of 25µl containing: 10X PCR Buffer, 3 mM $MgCl_2$, 1 mg/ml nuclease free BSA , 50 pmol of each primer, either primer for R1/R2 alleles, 10 mM of each dNTP, 0.125 U Taq polymerase and 2µl genomic DNA in the conditions: 95°C for 4 minutes, followed by 35 cycles of 95°C for 50 seconds, 58°C for 50 seconds, 72°C for 50 seconds and 72°C for 10 minutes for final extension.

The results were observed directly by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized by UV transillumination. A 426 bp product indicated the internal control and 151 bp fragment indicated the A or C allele for -592C/A position (**Fig 1**).

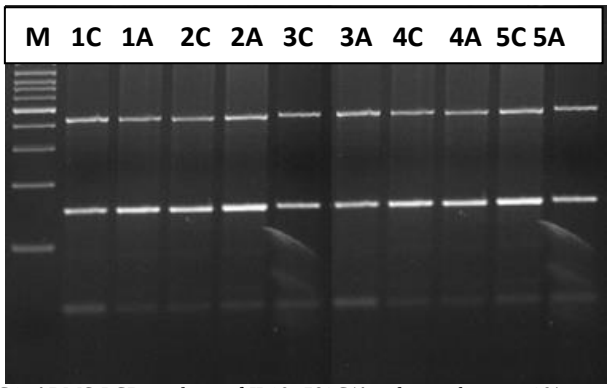

FIG1: ARMS-PCR products of IL10 -592C/A polymorphism on 2% agarose gel.

Lane M:100 bp ladder,
Lanes 1C & 1A, 2C & 2A, 3C & 3A, 4C & 4A, 5C &5A: heterozygous AC genotype (426 bp and 151 bp)

STATISTICAL ANALYSIS

All the statistical analyses were performed using the SPSS software for Windows version 20.0 (SPSS, Inc., Chicago, IL, USA) and Epi Info version 3.4.7 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Chi-square analysis was used to compare the genotype and allele frequency between asthma and control groups. Odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors and $p < 0.05$ was considered as statistically significant.

III. RESULTS

DEMOGRAPHIC CHARACTERISTICS

In this study, IL10 -592C/A polymorphism was genotyped in total of 964 subjects, including 483 healthy controls and 481 asthma patients. Various characteristics such as gender, age, disease duration, atopic status, IgE level, smoke exposure, family history, spirometry diagnosis, severity, BMI *etc.*were examined. The mean age for asthma patients was found to be 37 years and for healthy adults was 34 years. Also, the

females outnumbered the males in both the cases and controls. Mean disease duration was more than 9 years and 28% asthmatic patients had family history of asthma. Total serum IgE concentration (IU/ml) was assessed for 213 asthma patients and 125 control subjects and average of total IgE was higher in the asthmatics (2651.7 IU/ml) than the controls (776.1IU/ml). Spirometry data was only available for asthma patients so we are unable to apply the statistics (Table 1).

TABLE I: Demographic characteristics of the studied population.

PHENOTYPIC TRAITS	ASTHMATICS n (%)	CONTROLS n (%)	p
Gender Males Females	481 191 (39.7) 290 (60.3)	483 189 (39.1) 294 (60.9)	0.854
Age (Mean ±SD; years)	37.22±14.1 (range 18-57 years)	34.29±12.2 (range 18-60 years)	0.001*
Disease duration (years)	9.23	0	
Rhinitis Allergic rhinitis No rhinitis	 397 (82.5) 84 (17.5)	 0 483	0.000*
Allergy Allergic to atleast 2 provoking factors Non- allergic	 400 (83.5) 81 (16.8)	 0 483	0.000*
Smoking status Ever-smoker Non-smoker	 56 (11.6) 425 (88.5)	 0 483	0.000*
Spirometry data[a] FVC observed FVC predicted FEV1 observed FEV1 predicted FEV1/FVC observed FEV1/FVC predicted	(n=377) 2.94±1.1 2.23±1.6 2.74±1.1 2.61±0.66 68.92% 87.81%	nd	
Family history (n%)	135 (28%)	0	0.000*
BMI (kg/m^2) Underweight ≤18.5 Normal weight = 18.5–24.9 Overweight = 25–29.9 Obesity ≥ 30	23.7±2.1 16.6 21.15 26.85 33.7	23.96±12.3 17.4 21.3 26.7 30.8	0.606
IgE (IU/ml)[b]	2651.7	776.1	0.012*
Asthma severity[a] Normal Mild obstruction (FEV1%pred > 60%) Moderate obstruction (40% < FEV1%pred > 60%) Severe obstruction (FEV1%pred < 40%)	n=377 126 153 65 33	nd	

n- Number of subjects sampled, FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second,
BMI- Body Mass Index, nd- not determined, %- frequency, pred-predicted

[a]Spirometry test was conducted for 377 asthma patients

[b]IgE levels were confirmed for 213 asthma patients and 125 controls and given as average in IU/ml.

IV. PREVALENCE OF ALLELIC AND GENOTYPIC FREQUENCIES IN IL10 POLYMORPHISMS

The allelic frequencies in both the groups were equally prevalent among the cases (50%) and controls (50%) resulting in a non-significant association with OR=1.00, 95% CI (0.83-1.20) and p=1.00.

Comparing the genotypic frequencies in both the groups, it was observed that both the homozygous wild (CC) as well as homozygous mutant (AA) genotypes were completely absent from the studied population. However as both the asthma patients as well as the control groups showed heterozygous genotypes with 100% frequencies each, statistics cannot be applied for the genotypic frequenciesas well as phenotypic characteristics (Table 2).

TABLE II: Genotypic and allelic frequencies of IL10 -592C/A polymorphism in asthmatics and controls.

POLYMORPHISM	ASTHMAT	CONTR			
	481 (%)	483 (%)	χ^2	OR (95%	p
GENOTYPIC FREQUENCI					
CC	0 (0)	0 (0)		Ref (1.0)	
CA	481 (100.0	483 (100	----	--------	----
AA	0 (0)	0 (0)	----	--------	----
CA+AA	481	483	----	--------	----
ALLELIC FREQUENCIES					
C	481 (50.0)	483 (50.0)		Ref (1.0)	
A	481 (50.0)	483 (50.0)	0.00	1.00 (0.83-1.20)	1.00

DISCUSSIONS

The present study was conducted to reveal the impact of IL10 -592C/A polymorphism towards asthma in a North Indian population. Using allele-specific ARMS-PCR method, the allelic as well as the genotypic frequencies conferred a non-significant association with asthma (p>0.05) (Table 2).Furthermore, phenotypic characteristics werenot studied as both the alleles, C as well as A was equally distributed among the cases and the controls.

Asthma is caused due to airway inflammation as well as Th1/Th2 cytokines imbalance and IL10 suppresses the Th1 response, promotes B cell activation followed by the regulation of immunoglobulin class switching for IgE production [4,8]. An SNP

present at -592 positionleads to C to A substitution that lies within a negative regulatory region and results in low IL10 production [10]. As asthma is a chronic inflammatory disease, low levels of IL10 might be associated with the pathogenesis of the disease as the SNP precisely alters the binding site of transcription factors that could affect its production.

Studies performed in different human populations have suggested a diverse role of IL10 -592C/A polymorphism in the development of the inflammatory diseases. In accordance to our study, a case-control study conducted on the Korean population having 278 atopic asthmatics, 55 non-atopic asthmatics and 248 normal controls also found no difference in allele, genotype or haplotype frequencies of IL10 polymorphisms (-1082A/G, -819T/C, and -592A/C) [6]. In support of our study, a meta-analysis was carried out by combining eleven studies involving 2,215 asthma patients and 2,170 controls and it was found that the IL10 -592C/A polymorphism was not associated with asthma in the East Asian population [15].

Another case-control as well as a family-based study on North Indian population was conducted on 272 ethnically matched unrelated patients, 307 unrelated controls and 164 nuclear families. It was observed that the ATA haplotype of IL10 polymorphisms (-1082A/G, -819T/C, and -592C/A) was significantly associated, whereas GCC and a novel ATC haplotypes were not associated with asthma [16]. This controversial result may be due to more number of polymorphisms studied along with the experimental scenario. In addition, total serum IgE and the eosinophil counts are regulated by IL10 genotype in asthmatic. However, IL10 -592C/A polymorphism was not related to the susceptibility in asthma [17].

In contrast to our findings, a study conducted in the Iranian population reported that IL10 -592C/A genotypes were significantly associated with asthma among patients [18]. Another study conducted in the same population in a total of 100 asthmatic patients and 100 healthy controls revealed a significant association in both the genotypes and the alleles of the IL10 -592C/A gene polymorphism towards the disease and therefore suggested that the IL10 plays a crucial role in the pathogenesis of asthma [17]. The results of these population studies remain conflicting rather than conclusive. The association between asthma and the other polymorphisms within IL10 promoter were also reported by the researchers. These discrepancies may be due to the population size, phenotype heterogeneity, ethnic differences or various environmental parameters.

Varied results were obtained in a study conducted on 168 unrelated Chinese children and 53 age-matched healthy controls. Their result revealed that only allelic frequency of IL10 -592A/C was significantly different between Chinese children and their British counterparts (p=0.01) [19]. A meta-analysis carried out in a total of 4,716 asthma patients and 5,093 controls from the Asian population on IL10 polymorphisms and their haplotypes also found that IL10 polymorphisms are significantly associated with the disease. AC+AA vs CC genotype of -592A/C polymorphism again contributed significantly to the increased asthma susceptibility in adults [20]. However, the reference alleles of the IL10 -592 position were different in these studies.

CONCLUSIONS

This study concludes that the IL10 gene polymorphism at position -592C/A is not causing any risk towards asthma susceptibility in a North Indian population. However, complex interplay of genetic and environmental factors in asthma pathogenesis provides a promising field to explore the asthma related gene polymorphism studies which help in understanding the entities of this disease.

Conflict of Interest

The authors declare that they have no conflict of interest.

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PUBLICATIONS

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SYMPOSIUM AND WORKSHOP ATTENDED

1. Presented paper in the "1st International Conference on Human Implications of Biotechnology, ICHIB-2016"organized by Central university of Bihar and Jharkhand, Patna (Bihar) dated February 12-14, 2016.

2. Participated in6 days workshop on "Biorisk management trainer development program and curriculum development track" organized by Sandia National Laboratories, USA and Department of Biotechnology, P.U., Chandigarh (14-18 December, 2015).

3. Participated in6 days workshop on "Biorisk management and threat detection" organized by Sandia National Laboratories, USA and Department of Biotechnology, P.U., Chandigarh (3-9 April, 2014).