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Study on Association of Genetic Polymorphism of inflammatory gene IL-1βin susceptibility to Asthma: A Case Control study on population of Central Indian Region

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Abstract

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. It has been noticed that the occurrence of asthma has been increased dramatically. It affects both children and adults. Asthma is caused by multiple interacting genes, some having a protective effect and others contributing to the disease pathogenesis, Interleukin 1 beta which is an pro inflammatory cytokine has been seen associated with asthma by adhesion to vascular endothelium increase, growth factor for Th2 cells, B-cell growth factor, neutrophils chemo attractant, T cell and epithelial cell activation, eosinophil accumulation . On the basis of studies we can observe the association of interleukin 1 beta with asthma The patients and controls were from same age group with a male to female ratio 52:48. We have selected 160 asthmatic patients and 190 healthy population. As expected the asthmatic patients had differences in allelic variations of interleukin 1 beta gene as compared to healthy population.

Keywords: Asthma, IL-1Beta, Inflammation

Introduction

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. It has been noticed that the occurrence of asthma has been increased dramatically. It affects both children and adults. Asthma is regulated by a network of mutually interacting cytokines. It is seen that the development of asthma depends on environmental factors acting with a genetic predisposition. Asthma is a complex genetic disorder in which the mode of inheritance does not follow the classical Mendelian patterns and thus cannot be classified as autosomal, dominant, recessive or sex-linked. Genetic factors alone are not responsible in the pathogenesis of asthma but there is a close interaction with the environmental factors (Dr.Afia 2008). Cytokines plays a pathological role in asthma, particularly interleukin-1 gene family of cytokine proteins. Alleles of genes encoding immune/inflammatory mediators are associated with the disease.

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The airway inflammatory component of asthma is partly controlled by the genetic background of the patients [X.-Q. Mao., 2000]. Cytokine gene polymorphism could affect the serum levels of cytokine by influencing the transcriptional regulation. IL-1 is a major proinflammatory cytokine which could be seen in two forms of IL-1α and IL-1β [X.-Q. Mao., 2000]. In present investigation we studied –511 in the promoter region polymorphism of IL-1β gene (rs16944).

Material and Methods

Study Population

The study population consisted of 400 unrelated subjects comprising of 160 patients and 190 ethnically matched controls of central Indian population were included in this study. Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology Regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi. DNA Isolation and Quantification

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers. The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used.

Detection of Interlukin-1 Beta (IL1-β) Single Nucleotide Polymorphism via PCR-RFLP

The nucleotide position -511 in the promoter region of this gene has a single nucleotide polymorphism that results in change of nucleotide from cytosine (C) to thymine (T). The oligonucleotide sequence (primers) were designed to create a recognition site for the restriction enzyme AvaI in allele 1 (C at -511 position) but no restriction site in allele 2 (T at -511 position) of IL-1β gene.

Primer Sequences

The oligonucleotides sequences (primers) used were those described before. [Chaudhary et al., 2008]

IL1-β forward primer - 5' TGGCATTGATCTGGTTCATC 3'

IL1-β reverse primer - 5' GTTTAGGAATCTTCCCACTT 3'

PCR Mix

For each DNA sample 25 µl of PCR reaction mixture was prepared containing 5 µl template DNA (final concentration 100-200 ng/ µl), 2.5 µl of 10X Taq polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd., India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers, 0.2 µl of 5U/ µl of Taq DNA polymerase (final concentration 1U; Genetix Biotech Asia Pvt. Ltd.,India) and sterile water to set up the volume of reaction mixture to 25 µl.

Thermal Profile

Thermal profile used for the amplification of desired segment of gene was as follows: Initial denaturation at 95˚C for 2 min and 35 cycles of denaturation at 95˚C for 1 min, annealing at 54˚C for 1 min and extension at 74˚C for 1 min, followed by final extension at 74˚C for 10 min. [Chaudhary et al., 2008] PCR products were separated on 2% agarose gel (2% w/v, Sigma) using a 100 bp molecular weight (MW) marker to confirm the PCR product size of 304 bp.

Restriction Digestion by AvaI

The C to T transition in promoter region of IL1- β gene when amplified by PCR was than incubated with AvaI restriction enzyme (New England Biolabs,USA). Digestion of the amplified 304 bp PCR product gave two fragments in PAGE of 190 bp and 114 bp respectively if the product was excisable by Ava I. Depending on digestion pattern, individuals were scored as genotype CC when homozygous for presence of Ava I site, genotype TT when homozygous for absence of Ava I site and genotype CT in case of heterozygosity.

Results and Discussion

The result of the investigation depends on test for asthmatic people utilizing certain kits and certain questionnaire designed. The patients and controls were from same age group with a male to female ratio 52:48. Their demographic characteristics , distribution on the basis of family history , history of allergy and seasonal variations in symptoms are given in Table 1.

Table 1: Characteristics of population sample

Prevalence of asthma symptoms : (n=160)		Percentage
By Gender	Male	**52**
	Female	**48**
Family History :		
No Family History		**62**
Of Asthma		**32**
Of any other allergic condition (Eczema, allergic rhinitis)		**6**

Anthropometric results

We have selected 160 asthmatic patients and 190 healthy population .The descriptive data and comparison of anthropometric parameters of asthmatic patients versus controls are presented in Table 5(b). As expected the asthmatic patients had markedly higher levels of weight of women (P=0.0024), Men (P=0.0157) and BMI of Women (P=0.0388), Waist circumference in women (P<0.0001), WHR in Women (P<0.0001) and WHR in Men (P=0.0147)

Table 2: Characteristics

Characteristics	Cases	Controls	P-value
n(Men/Women)	160(83/77)	190(99/91))	
Age(years)	52.5±12.5	53.0±14.2	0.7100
Height(m)	160.50±13.40	162.2± 12.000	0.1815
Weight (Kg)			
Women	62.5 ±5.70	60 ± 4.50	0.0024 **
Men	68±5.60	66.0±7.1	0.0157*
BMI (kg/m^2)			
Women	26.4±3.1	25.1 ± 4.3	0.0388*
Men	24.6±4.7	24.1± 5.1	0.4301

Table 3: Fisher Exact Test values of IL-1β 511 C/T polymorphism

IL-1β GENOTYPE	CASE N= 160 n %	CONTROL N=190 n %	P Value	ODDS RATIO(95% CI)
CC	15 09.37	3920.52	0.0046**	0.4005(0.2117- 0.7579)
CT	62 38.75	80 42.11	0.5450	0.8699 (0.5663- 1.336)
TT	83 51.85	71 37.36	0.0070*	1.807 (1.178- 2.77)
ALLELES				
C	92 28.75	158 41.57	0.0005***	0.5670 (0.4131- 0.7782)
T	22871.25	222 58.42		1.764 (1.285 -2.421)
CARRIGE RATE				
C	7748.12	119 62.63	0.0417*	0.6738 (0.4672- 0.9719)
T	145 90.62	15179.47		1.484 (1.029 - 2.141)

*denotes the significant association between case and control
N – Number of individuals in study group %- Genotype allele frequency and carriage rate expressed in percentage

Detection of genetic polymorphism in IL-1β

The nucleotide position –511 in the promoter region of IL-1β gene has a SNP (rs16944) that results in change of nucleotide from cytosine (C) to thiamine (T). The PCR products when digested by restriction enzyme AvaI, generated allele 1 (C at –511 position) but no restriction site in allele 2 (T at –511 position) of IL-1β gene PCR product size is 304 bp. After AvaI digestion two fragments of 190 bp and 114 bp were generated.

Overall distribution of IL-β-511 genotypes was significantly different in healthy control (HC) group as compared to disease group. HC group showed a significant increase in 'CC' genotype as compared to Patients of asthma (15% Vs 39%). Similarly, 'TT'

genotype was present in significantly lower frequency in Healthy Control (HC) as compared to asthma patients group (83% vs. 71%), heterozygous was slightly different in case as compare to control. An odds ratio of 0.4005 in asthma group respectively for 'CC' genotype indicated a protective effect of this wild type genotype in our population whereas an odds ratio of 1.807 of asthma patients group respectively indicated a harmful effect and positive association of this mutant genotype 'TT' with the disease susceptibility.

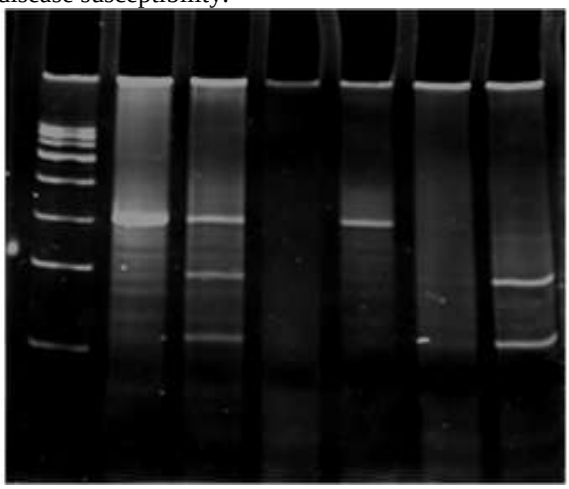

Fig. 1: Representative gel picture of IL1β 511 C<T polymorphism

Lane 1 : 100 bp MW marker**; Lane 2 & 5** Genotype CC **; Lane 3:** Genotype CT; **Lane 4&6:** Negative control;**Lane 7:**Genotype TT

Heterozygous CT showed odds ratio of 0.8699 which indicates that heterozygous no effect in disease susceptibility. Overall allele 'C' was found in significantly lower frequency in disease group as compared to HC group whereas allele 'T' was present in significantly high frequency in the disease group (χ^2 =12.75, P value= 0.004*). An odds ratio of 1.764 of 'T' allele showed stronger association of overall "T' allele frequency in asthma susceptibility. Carriage rate of allele 'C' was significantly higher in HC group whereas carriage rate of allele 'T' was high in disease group (χ^2 =4.481, P value 0.0343*). The carriage of "T' Allele revealed odds ratio of 1.484 which indicates association of the "T' allele carriage with asthma susceptibility in our population. The frequencies of TT genotype and T allele were significantly higher in patients than controls and associated with high risk (p =0.0070; OR= 1.807, 95% CI 1.178- 2.77 and p=0.0001; OR=1.764, 95% CI 1.285 -2.421 respectively). On the other hand, the CC genotype and C allele had low risk (p=0.0046; OR=0.4005, 95% CI 0.2117- 0.7579 and p=0.0005; OR=0.5675, , 95% CI 0.4131- 0.7782 respectively).

Conclusion

IL-1β cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. Gene-by-environment factor seems to be a key process in the development and expression of asthma [S. McLeish et al., 2007]. The airway inflammatory component of asthma is partly controlled by the genetic background of the patients [X.-Q. Mao et al., 2000]. Cytokine gene polymorphism could affect the serum levels of cytokine by influencing the transcriptional regulation. IL-1 is a major proinflammatory cytokine which could be seen in two forms of IL-1α and IL-1β [X.-Q. Mao et al., 2000, K. F. Chung, 1999]. These molecules are structurally related and they share a similar profile of functions by binding to the same receptors with different affinity. The natural inhibitor of IL-1, IL-1 receptor antagonist, mediates its effect by binding IL-1 type I receptor and blocking IL-1 binding on target cells. The IL-1 gene linkage to asthma has been reported in more than one study [H. Hakonarson, 2001]. The human genes for IL-1β, IL-1α, their receptors, and the IL-1 receptor antagonist are clustered on chromosome 2 (q14–q21) [K. F. Chung., 1999, X.-Q. Mao et al., 2000, C. A. Dinarello., 2000]. Previous study in vindhyan region associate IL-1 Beta polymorphism with diabetes type 2. (Tripathi et al., 2015) and Our finding associate IL-1 Beta polymorphism with asthma susceptibility.

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