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IFN-γ +874 and IL-4 -590 Polymorphisms and Asthma Susceptibility in North West of Iran

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ABSTRACT

Background: *Recent studies have documented an association between some cytokines' gene polymorphisms and chronic inflammation of the respiratory tract which leads to asthma susceptibility. This study was conducted to investigate if there were any differences in IFN-γ +874 (A/T) and IL-4 -590 (C/T) single nucleotide variations in asthmatic patients compared to normal controls among West Azerbaijani population.*

Materials and Methods: *IFN-γ +874 (A/T) and IL-4 -590 (C/T) polymorphisms were amplified by ASO-PCR and RFLP-PCR from genomic DNA of 173 individuals including 64 asthmatic patients and 109 control subjects from West Azerbaijani population.*

Results: *The allele or genotype frequencies of IFN-γ +874 A/T in patients were not different from those of controls (p>0.05). The differences between allelic or genotypic frequencies of IL-4 -590 C/T in patients and controls were not statistically significant (p>0.05).*

Conclusion: *These findings showed that IL-4 -590 (C/T) and IFN-γ +874 (A/T) polymorphisms were not associated with asthma susceptibility.* ***(Tanaffos2010; 9(4): 22-27)***

Key words: *Polymorphism, Asthma, Interferon- γ (IFN-γ), Interleukin-4 (IL-4)*

INTRODUCTION

Asthma etiologies are poorly understood. The mechanisms that lead to the development of asthma are complex and vary among populations and even from individual to individual. Asthma is an important public health problem and its prevalence has increased over the last decade worldwide (1-4). Asthma has several risk factors including environmental factors and genetic background. The nature of genetic risk factors is unknown (5-7). It has been found that some of the candidate genes which are located on chromosomes 5q31-33, 6, 11, 12, and 13 are associated with asthma susceptibility in different populations, and some other positions on chromosomes 2, 7, 14, 17, 19, and 21 may also play a role in asthma susceptibility (8). Results from a large

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number of studies have shown an association among cytokines and cytokine gene polymorphisms and asthma susceptibility in different ethnic populations (9). An imbalance between TH1 and TH2 cytokines leads to the development of inflammatory responses of the immune system (10). Chronic inflammation of the air-ways is associated with hyper-responsiveness which may result in asthma (11).

The aim of this study was to determine if there were any associations between IFN-γ +874 (A/T) and IL-4 -590 (C/T) polymorphisms and asthma susceptibility.

MATERIALS AND METHODS

A total of 173 subjects including 64 asthmatic patients (aged 44.56±15.85 yrs; 28 males, and 36 females) and 109 control subjects entered the study. Asthmatic patients were diagnosed and sequentially selected among patients referred to Imam Hospital affiliated to Urmia University of Medical Sciences. The inclusion criteria were: 1) age older than 15 years old, 2) history of asthma, 3) non-smoker, 4) spirometry test findings of FEV1/FVC<0.7, and FEV1<70% predicted and 15% increase in FEV1 after bronchodilator inhalation, and 5) asthma must be clinically confirmed by a pulmonologist. Normal subjects were randomly selected from the same ethnic group among participants in the genetic counseling sessions taken place in genetic department of Urmia University of Medical Sciences. They were selected based on their past medical history and exclusion of any specific disorders such as genetic diseases, history of atopy, or asthma. After taking an informed written consent, DNA was extracted from 3-5 ml whole peripheral blood collected with EDTA as anticoagulant using the salting out method (12).

IFN-γ +874 (A/T) genotyping:

ASO-PCR was carried out for IFN-γ +874 (A/T) genotyping using common primer 5′-tca aca aag ctg ata ctc ca-3′, T allele primer 5′-ttc tta caa cac aaa atc aaa tct-3′ and A allele primer 5′-ttc tta caa cac aaa atc aaa tca-3′, under a cycling program which consisted of 10 cycles (denaturation at 94°C for 30 sec, annealing at 62°C for 50 sec, and extension at 72°C for 40 sec), and 20 cycles (denaturation at 94°C for 20 sec, annealing at 56°C for 50 sec, and extension at 72°C for 40 sec) (13).

IL-4 -590 (C/T) genotyping:

RFLP-PCR was performed for IL-4 -590 (C/T) genotyping using forward primer 5'-taaacttgggagaacatggt-3' and reverse primer 5'-tggggaaagatagagtaata-3', with PCR program including 35 cycles (denaturation at 95°C for 50 sec, annealing at 53°C for 50 sec, and extension at 72°C for 50 sec (14).

Each PCR reaction was carried out in a 20 µl solution containing 100 ng of genomic DNA, 1x reaction buffer 10 pmol of each primer, 200 µmol of each dNTPs, 0.5 unit of Taq DNA polymerase, and 1.5 mmol $MgCl_2$. IL-10 and IFN-γ amplicons consisted of 550 and 261 bp fragments, respectively. IL-4 amplicons contained 195 bp fragments and after digestion with AvaII enzyme at 37°C for 2 hours produced two fragments of 177 and 18 bp. In heterozygote IL-4 -590 (C/T) three fragments (18,177, and 195bp), in homozygote IL-4 -590 (C/C) two fragments (177 and 18 bp) and in homozygote (T/T) undigested product should be detected. Electrophoresis of PCR products and digested fragments was carried out on 3% agarose gel, and presence or absence of fragments were visualized by UV transilluminator. The allelic and genotypic frequencies of polymorphisms were determined via direct counting and dividing by the number of chromosomes and the number of subjects

respectively. Allelic and genotypic frequencies in the studied cases were compared with controls using χ^2 test. Data were tested for their fit to Hardy-Weinberg equilibrium. The minimum sample size determined according to the two-sided test, power (1-β): 90% level of significance α: 5% was 38. The SPSS ver. 16.0 software were used to calculate the χ^2 value, the odds ratio (OR), and 95% confidence interval (CI). P-value less than 0.05 was considered statistically significant. The ethics committee of Urmia University of Medical Sciences (West Azerbaijan, Iran) approved this study.

RESULTS

Demographic characteristics of cases and our study results are summarized in Tables 1 and 2. The frequencies of IFN-γ +874 A and T alleles were 0.51 and 0.49 in patients and controls, respectively. IFN-γ +874 AA, AT and TT genotype frequencies were 17(26.56%), 31(48.44%) and 16(25%) in patients and 26(23.85%), 59(54.13%), and 24(22.02%) in controls, respectively. The allele or genotype frequencies of IFN-γ +874 A/T in patients were not different from those of controls (p >0.05, Table 2). The frequencies of IL-4 -590 C and T alleles were 0.86 and 0.14 in patients, and 0.82 and 0.18 in controls, respectively. IL-4 -590 CC, CT and TT genotype frequencies were 46(71.88%), 18(28.13%) and 0(0%) respectively, in the patients and 42(64.62%), 23(35.38%) and 0(0%) respectively, in the controls. The difference in allelic or genotypic frequencies of IL-4 -590 C/T between the patient and control groups was not statistically significant (p >0.05, Table 2).

Table 1. Demographic characteristics and types of therapy of patients

Variables		No (%)
Gender	Males	28(43.75)
	Females	36(56.25)
Family history	Yes	28(43.75)
	No	36(56.25)
Drug therapy	Monotherapy	36(56.25)
	Combined drug therapy	28(43.75)

Table 2. Allelic and genotypic frequencies of IFN-γ +874 (A/T) and IL-4 -590 (C/T) in asthmatic patients and the control group. Totally 64 patients with asthma were genotyped for IFN-γ +874 (A/T) and IL-4 -590 (C/T); 109 controls were genotyped for IFN-γ +874 (A/T), and for IL-4 -590 (C/T) it was possible to genotype 65 controls.

Polymorphism	Patients f(%f)	Controls f(%f)	OR(95% CI)	χ^2	P-Value
IFN-γ +874					
A	65(50.78)	111(50.92)	0.995(0.643-1.539)	6E-04	0.98
T	63(49.22)	107(49.08)	1.005(0.65-1.556)	6E-04	0.98
A/A	17(26.56)	26(23.85)	1.155(0.569-2.345)	0.159	0.691
A/T	31(48.44)	59(54.13)	0.796(0.429-1.477)	0.523	0.469
T/T	16(25)	24(22.02)	1.181(0.572-2.437)	0.202	0.653
IL-4 -590					
C	110(85.94)	107(82.31)	1.314(0.671-2.572)	0.636	0.425
T	18(14.06)	23(17.69)	0.761(0.389-1.49)	0.636	0.425
C/C	46(71.88)	42(64.62)	1.399(0.664-2.949)	0.784	0.376
C/T	18(28.13)	23(35.38)	0.715(0.339-1.506)	0.784	0.376
T/T	0(0)	0(0)			

f: frequency

DISCUSSION

In this study, we analyzed the allelic and genotypic frequencies of IFN-γ +874 A/T, and IL-4 -590 C/T polymorphisms in asthmatic patients and controls in West Azerbaijani population (North-West of Iran). Several studies have revealed an association between the levels of cytokine production and polymorphisms of IL-10 -1082 A/G, IFN-γ +874 A/T and IL-4 -590 C/T. The majorities of polymorphisms are single nucleotide polymorphisms located within enhancer, promoter or other regulatory sequences of cytokine genes (13,14). It has been documented that racial and ethnic differences among populations strongly influence on cytokines' polymorphisms (15).

Asthma, as a common respiratory disorder is caused by acute/chronic bronchial inflammation (16). It has been demonstrated that cytokines' genetic variations, gene-gene and gene-environmental interactions could influence pathogenesis of asthma with changing the T_H1/T_H2 balance (16). IFN-γ plays a critical role in the development of T_H1 subtype. Transcription regulation of IFN-γ gene is under control by several transcription factors that bind to the promoter or inducer region. IFN-γ +874 (A/T) polymorphism has three genotypes (phenotypes) as T/T (high production), T/A (intermediate production) and A/A (low production). There is a significant association between presence of +874T allele and increased IFN-γ production (13). Intermediate production genotype (A/T) is more common in normal individuals (13). Our finding showed that IFN-γ +874 A/T allelic or genotypic frequencies in asthmatic patients were not statistically different from those of controls; and implied that IFN-γ could not be a significant risk factor in asthma predisposition. It is difficult to explain why there was no association between the IFN-γ +874 A/T polymorphism and asthma predisposition. Environmental factors in our population might be different and such factors play a significant role in the pathogenesis of asthma. In another study in Iran, IFN-γ polymorphism was evaluated in asthmatic cases and compared to that of controls (16). Daneshmandi et al. (2008) suggested that IFN-γ +874 (A/T) polymorphism was not a risk factor for asthma (16). IL-4 showed a wide range of biological functions and was defined as the main regulator of allergic responses. IL-4 and IL-5 contribute in the production of IgE as well as increased production of IgE involved in the pathogenesis of asthma (17). In the previous studies there were controversies regarding the association between IL-4 -590 C/T polymorphism and the production level of IgE or asthma development (18-20). Noguchi et al. (1998) found an association between T allele at IL-4 -590 position and development of asthma in a family study of Japanese population (20).

According to our study, allelic and genotypic frequencies of IL-4 -590 C/T polymorphisms were not significantly different between cases and controls; IL-4 -590 T/T genotype frequency in our population was approximately zero. However, we could not find an association between this polymorphism and asthma. Results of some investigations support our finding (21,22), but others do not (14,19,20,23-25). In some other centers in Iran, IL-4 -590 T/C single nucleotide polymorphisms were analyzed in cases with asthma and controls (16,26). Daneshmandi et al. in 2008 suggested that IL-4 -590 T/C polymorphism was not a risk factor for asthma (16). Kamali-Sarvestani et al. in 2007 reported that the IL-4 –590 C/T nucleotide variations were associated with asthma susceptibility but not with asthma severity (26). Although in the present study we did not find any association between the polymorphic regions of IFN-γ +874 and IL-4 -590 and asthma, we could not exclude other polymorphisms within these genes or their receptors ,

as well as other cytokines' gene variations (such as IL-10 and IL-2). These findings indicate that it is necessary to analyze other haplotypes concerning candidate genes and individual specific genetic variations in a large cohort of well-defined ethnic groups as well as comprehensive functional analysis of gene and protein functions in association with diseases like asthma. Results of several studies showed wide range of variations because many factors may explain controversial findings such as: i) design of study ii) sample size, iii) case-control study, iv) family linkage study, v) ethnicity differences, and vi) diagnosis of the asthmatic patients using definite criteria. The Limitation of our investigation was that cases were not precisely matched in terms of the number of contributors, and gender. Considering the possibility of producing individualized drugs based on the genetic polymorphisms in the near future, the profiling of various cytokines' polymorphisms in individuals may help clinicians in selecting appropriate prophylactic measures and treatments based on the genetic background of patients.

CONCLUSION

Our results suggest that IFN-γ +874 A/T and IL-4 -590 C/T polymorphisms are not associated with asthma susceptibility.

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