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Research Article

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Evaluation of C-590T Promoter of IL-4 Gene Polymorphisms in Patients with Sinonasal Polyposis

Soheila Nikakhlagh[1], Ata Ghadiri[2], Nader Saki[1*], Maryam Kardoni[3] and Ali Konari[4]

[1]Associate Professor of Otolaryngology, Hearing & Speech Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Assistant Professor of Immunology, Cancer Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Assistant Professor of Otolaryngology, Hearing & Speech Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
**Email: ahvaz.ent@gmail.com*

ABSTRACT

Despite different studies about the role of cytokines in development of nasal polyps, there are only few studies about the interleukin-4 and the role of this cytokine is not yet clearly known. Hence in this study, the genetic polymorphism in interleukin-4 which is not down-regulated by anti-inflammatory therapeutics was assessed in nasal polyposis patients in comparison with control group. In this cross-sectional comparative study, 152 consecutive subjects attending to a tertiary health care center in 2015 including 76 nasal polyposis patients and 76 control subjects were enrolled. Five milliliters blood sampling was done by single trained subject in sterile conditions and it was stored in EDTA-containing vials and froze in –20 Celsius to extract DNA with real-time PCR. DNA was extracted by chloroform phenol method and the Tag man real time was used for genotyping of C-590 promoter location in patients and subjects by special probes using the Step-one real time PCR (ABI Company). The genotypes were compared across the groups. Chi-Square and Independent-Sample-T tests were used and were considered statistically significant at P values less than 0.05. There were no statistically significant differences (P= 0.404) between genotype in patients in two groups. The age and gender were not significantly related to the genotype in subjects in two groups (P > 0.05). Totally it may be concluded that genetic polymorphism in interleukin-4 gene location in 590 promoter locus is not related to nasal polyposis. However further studies should be carried out to attain more definite results and decrease the controversies about the role of inflammatory cytokines in pathogenesis of nasal polyposis.

Keywords: Genetic polymorphism, Interleukin-4, Nasal polyposis

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INTRODUCTION

Polyp is a mucosal edematous membrane seen as a prominent lesion. The polyps are usually pedanculated and are originated from ethmoidal sinus in upper respiratory tract presenting in nasal cavity via middle meatus leading to

airway obstruction. The stroma is very edematous with different concentration of inflammatory cells. The polyps are usually accessible for histological and immunological studies resulting in numerous studies about them. These polyps are usually accompanied with rhinitis and asthma; but the role of allergy in etiology and pathogenesis of nasal polyps is controversial (1, 2). There are different hypotheses about the basic mechanism of nasal polyp generation including chronic infection, Aspirin hypersensitivity, aerodynamic changes, epithelial gene disturbance, inhalational or oral allergens, and sodium ion absorption changes. Some hereditary factors may also be related. High level of inflammatory mediators in nasal polyps is common and significant showing the role of chronic stable inflammation as a cardinal factor in polyp generation (3, 4). Nasal polyps are recurrent despite antibiotic and steroid treatment and use of surgical methods (5). Nasal polyposis has a prevalence rate of two to five percent with high socioeconomic burden (6, 7). During recent decades, different studies are performed about the etiology of nasal polyps; but definite cause is not known. Despite the role of atopy in nasal polyposis, the exact role of allergy is not yet known. Nowadays the nasal polyposis is considered as a multifactorial entity presenting with chronic inflammation in mucosal membrane of paranasal sinuses as a consequence of chronic rhinosinusitis (8, 9). A combination of allergic, infectious, anatomical, and genetic factors are known as cause of nasal polyposis (10). Currently the cytokines and T-helper cells are known as main contributing factors in many diseases such as hepatic injuries, rheumatoid arthritis, asthma, etc (11, 12). Also increased levels of different cytokines in serum in the patients with allergic rhinitis are seen and the association between severity of symptoms and cytokines level are studied (13). Nasal epithelium is the first site of exposure to inspirational antigens and may have a pivotal role in pathogenesis of allergic diseases. It seems that nasal and paranasal epithelium cells are contributing for nasal cavity polyposis with secretion of various cytokines (14). Despite different studies about the role of cytokines in development of nasal polyps, there are only few studies about the interleukin-4 and the role of this cytokine is not yet clearly known. On the other hand regarding the long-term treatment courses with oral and inhalational corticosteroids, the level of inflammatory factors is not reliable in the patients. Hence in this study, the genetic polymorphism in interleukin-4 which is not down-regulated by anti-inflammatory therapeutics was assessed in nasal polyposis patients in comparison with control group.

MATERIALS AND METHODS

In this cross-sectional comparative study, 152 consecutive subjects attending to a tertiary health care center in 2015 including 76 patients with nasal polyposis and 76 control subjects without history of chronic sinusitis, allergy and asthma were enrolled. The exclusion criteria were autoimmune disease, Immunoglobulin-E deficiency, neoplastic diseases, and cystic fibrosis. All patients were treated by endoscopic surgery at the otolaryngology department of Imam hospital. The diagnosis of chronic sinusitis with polyposis was base on the history, clinical examination, nasal endoscopy and CT scan. The sampling was from all racial groups without consideration of drug history in patients.

This study was approved by Local ethical committee (REC.ajums.ac.ir 1394.424) and the informed consent form was fulfilled by all enrolled subjects. The five milliliter blood sampling was done by single trained subject in sterile conditions and it was stored in EDTA-containing vials and froze in – 20 Celsius to extract DNA with real-time PCR. DNA was extracted by chloroform phenol method and the Tag man real time was used for genotyping of C-590 promoter location in patients and subjects by special probes using the Step-one real time PCR (ABI Company). The data for each patient were recorded in checklists. Data analysis was performed by SPSS (version 20.0) software [Statistical Procedures for Social Sciences; Chicago, Illinois, USA]. Chi-Square and Independent-Sample-T tests were used and were considered statistically significant at P values less than 0.05.

RESULTS

The mean (standard deviation) age was 41.95 (11.89) years and 31.89 (11.24) in case and control groups, respectively (P= 0.0001). Fifty-four patients (71.1%) and 39 subjects (51.3%) were male in case and control groups, respectively (P= 0.013).

There were no statistically significant differences (P= 0.404) between genotype in patients in two groups (Figure 1). As shown in Tables 1 and 2; the age and gender were not significantly related to the genotype in subjects in two groups (P > 0.05).

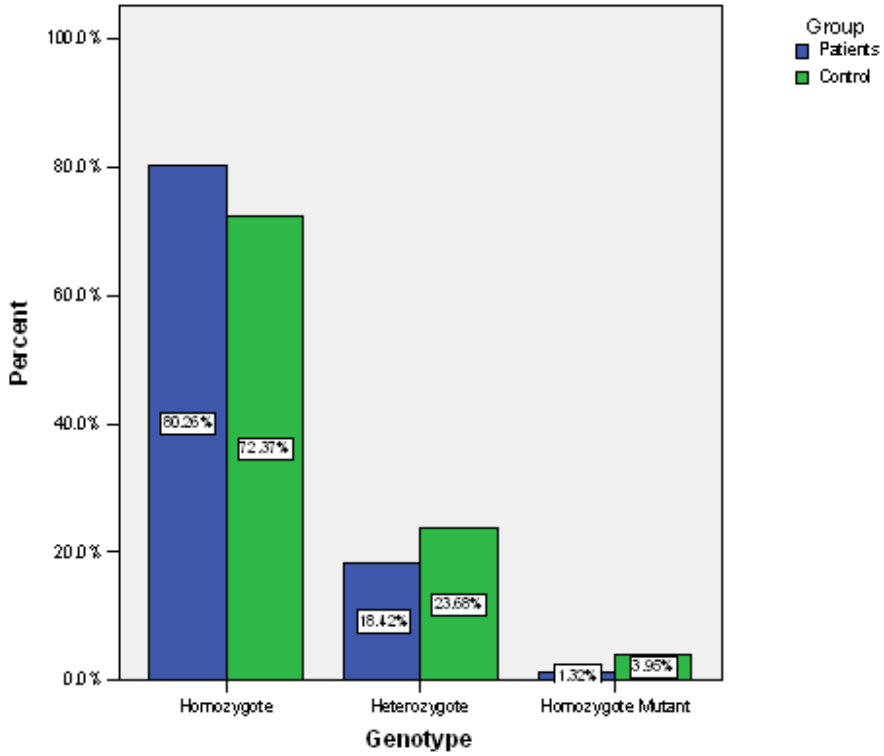

Figure 1. Distribution of genotypes in patients in two groups

Table 1. Age distribution according to genotypes in two groups

Group	Genotype	Mean	Standard Deviation
Patients*	Homozygote	41.57	11.73
	Heterozygote	42.50	12.75
	Homozygote Mutant	57.00	-----.
Control*	Homozygote	31.98	10.45
	Heterozygote	32.17	14.37
	Homozygote Mutant	28.67	4.73

** Age was not significantly related to the genotype in patients in two groups (P > 0.05).*

Table 2. Gender distribution according to genotypes in two groups

Group	Gender	Homozygote	Heterozygote	Homozygote Mutant	Total
Patients*	Male	44 (81.5%)	9 (16.7%)	1 (1.9%)	54 (100%)
	Female	17 (77.3%)	5 (22.7%)	------	22 (100%)
	Total	61 (80.3%)	14 (18.4%)	1 (1.3%)	76 (100%)
Control*	Male	27 (69.2%)	10 (25.6%)	2 (5.1%)	39 (100%)
	Female	28 (75.7%)	8 (21.6%)	1 (2.7%)	37 (100%)
	Total	55 (72.4%)	18 (23.7%)	3 (9.3%)	78 (100%)

** Gender was not significantly related to the genotype in patients in two groups (P > 0.05).*

DISCUSSION

In this study, the genetic polymorphism in interleukin-4 gene location was assessed in the nasal polyposis patients in comparison with control subjects. It was seen that there were no statistically significant differences between case and control subjects for their genotypes and also the age and gender were not significantly related to the genotype status in subjects in two groups.

In a study by Farhadi *et al.* (15) both immunoglobulin-E level assessment and real-time PCR methods were used among 75 subjects including nasal polyposis and control groups. The frequency rate of interleukin-4 gene presence was 44.7 percent and 18.9 percent in case and control groups, respectively showing significant difference. However

in their study, the interleukin-10 and 12 and the P40 and P35 fractions were same across the groups. This matter shows that Th1/Th2 disequilibrium has a pivotal role in nasal polyp pathogenesis. However the chronic inflammation is the main contributing factors. The study by Ekinci and colleagues (16) demonstrates that Eotaxin-1 gene polymorphism was significantly more among those with nasal polyposis in comparison with control subjects. However it was not a significant difference as well as our finding about the Interleukin-4.

In a study by Allen *et al*. (17) the polyp tissue from 134 patients were assessed for interleukin-8 levels with immunohistochemical and radioimmunoassay methods showing increased levels in all subjects in epithelial and inflammatory cells. Their study revealed important role of interleukin-8 in pathogenesis of nasal polyps. However this role was not found for interleukin-4 in our study. In the study by Kosagi and colleagues (18) the polymorphism in 174GC allele of interleukin-6 was assessed in 45 and 63 polyposis patients with and without asthma in comparison with 45 and 81 patients with asthma without polyposis and control subjects. The GG was the most common genotype in case group which was seen in only less than 40 percent of control subjects showing significant difference.

Joki Erkkila *et al*. (19) assessed 245 patients with nasal polyposis with and without asthma for interleukin-A1 and it was seen that homogenous allele of G was significantly more in subjects with concomitant asthma and nasal polyposis. In the study by Yea and colleagues (20) the polymorphism in C-590T promoter locus of interleukin-4 was assessed in 106 nasal polyposis patients with and without asthma and 70 control subjects. It was seen that T allele had protective role in comparison with CC allele and the CC allele was increased in case subjects.

Totally, according to the obtained results, it may be concluded that genetic polymorphism in interleukin-4 gene location in 590 promoter locus is not related to nasal polyposis. However further studies should be carried out to attain more definite results and decrease the controversies about the role of inflammatory cytokines in pathogenesis of nasal polyposis.

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Authors' Contributions
All authors had an equal role in the design, work, statistical analysis and manuscript writing.

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