

TNF- α and IL-1 β Cytokine Gene Polymorphism in Patients with Nasal Polyposis

Nazal Polipozisli Hastalarda TNF- α ve IL-1 β Sitokin Gen Polimorfizmi

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Original Investigation
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Abstract

Objective: Nasal Polyp (NP) is a benign mass of the paranasal sinuses that protrudes into the nasal cavity. The exact underlying pathogenesis is not known. In this study we aimed to determine the genetic susceptibility of NP formation in relation to TNF- α -308 and IL-1 β -511 promoter region gene polymorphisms.

Methods: A total of 71 patients with NP with asthma (n=21) or without asthma (n=50) were taken as the study group, and 91 healthy volunteers were taken as the control group. Blood was gathered into EDTA-containing tubes, and patient DNA was extracted. The polymorphisms of the IL-1 β and TNF- α cytokine genes were analyzed using real time polymerase chain reaction.

Results: The GG genotype in the TNF- α -308 region and the CC genotype in the IL-1 β -511 region were found to be risk factors for NP formation (OR: 9.2, p=0.007 and OR: 33.3, p=0.001, respectively). Regarding allelic frequencies, the G allele at the TNF- α -308 promoter region was a risk factor for NP formation (OR: 6.06, p<0.001).

Conclusion: TNF- α GG genotype in the -308 promoter region and the IL-1 β CC genotype in the -511 region are genetic risk factors for NP formation.

Keywords: Nasal polyp, cytokine, polymorphism, genetic, TNF- α , IL-1 β

Öz

Amaç: Nazal polipler (NP), paranasal sinüslerin nazal kaviteye uzanım gösteren benign oluşumlardır. NP oluşumuna neden olan etken tam olarak bilinmemektedir. Bu çalışmada NP oluşumunun genetik yatkınlığını TNF- α -308 ve IL-1 β -511 gen polimorfizm analizi ile incelemeyi hedefledik.

Yöntemler: Astımla birlikte olan ya da olmayan 71 NP hastası (50 astım olmayan, 21 astımlı hasta) ve sağlıklı 91 gönüllü çalışmaya dahil edilmiştir. EDTA içeren biyokimya tüplerine kan alınmış ve DNA ekstraksiyonu gerçekleştirilmiştir. Gerçek zamanlı polimeraz zincir reaksiyon tekniği kullanılarak TNF- α ve IL-1 β sitokin gen polimorfizmleri analiz edilmiştir.

Bulgular: TNF- α -308 bölgesinde GG genotipi ve IL-1 β -511 bölgesindeki CC genotipi NP oluşumu için risk faktörü olarak bulunmuştur (sırasıyla Odds oranı:9.2, p=0.007 ve Odds oranı:33.3, p=0.001). Allelik frekanslarda TNF- α -308 bölgesinde G aleli NP oluşumu için risk faktörüdür. (Odds oranı: 6.06, p<0.001)

Sonuç: TNF- α -308 promoter bölgesindeki GG genotipi ve IL-1 β -511 bölgesindeki CC genotipi NP oluşumu için genetik risk faktörleridir.

Anahtar kelimeler: Nazal polip, sitokin, polimorfizm, genetik, TNF- α , IL-1 β

Introduction

Chronic rhinosinusitis with nasal polyps (NP) is a disease of the paranasal sinuses and the nasal cavity and is distinguished by extracellular edema and abundant inflammatory cells. It might cause a certain degree of morbidity such as nasal obstruction, rhinorrhea, and anosmia (1). Although the underlying pathogenesis has been presumed to be infections, allergies, or immunological diseases, the exact pathogenesis has not been fully elucidated (1). Not all patients with chronic rhinosinusitis or allergic

rhinitis have NPs; therefore, genetic predisposition such as glutathione-S-transferase genetic polymorphism, increased gene expression of chemokines, metalloproteinases, growth factors, and increased expression of leukotriene receptor genes are thought to be the underlying cause of NP pathogenesis along with accompanying environmental factors (1-3).

The histopathological examination of NP demonstrates epithelial proliferation, glandular hyperplasia, basal membrane thickening, edema, fibrosis,



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and cellular infiltration (4). The mediators secreted from inflammatory cells stimulate a cascade of reactions that result in ongoing inflammation, mucosal edema, and subsequent NP development. When compared to antrochoanal polyps, the nasal polyps have many more submucous glands and much more eosinophilic infiltration (5, 6).

Many cytokines and inflammatory mediators show increased concentrations in NP tissue. Tumor necrosis factor (TNF)- α and Interleukin-1 (IL-1) are pro-inflammatory cytokines that take part in the signaling cascades that are involved in the pathogenesis of NP. They have synergistic effects such as recruitment of eosinophils by upregulating adhesion molecules during NP formation. Single nucleotide polymorphisms (SNPs) in these pro-inflammatory mediators have been studied for genetic susceptibility to NP development, but the literature findings regarding the dominant genotype or allele causing NP formation are conflicting. Some authors claim that the TNF- α GA polymorphism in the -308 promoter region is a risk factor for NP development, whereas others have found that the A allele frequency is a risk factor (7-9). Some authors have found no relationship between the TNF- α GA polymorphism and NP development (4, 10).

Because the number of previous studies is limited, and due to the conflicting results, we aimed to investigate the polymorphisms of the IL1- β and TNF- α genes in NP patients in a Turkish population.

Methods

Local ethics committee approval was acquired for the study, and signed informed consent was obtained from all NP patients and control group participants. The study group consisted of 71 patients with NP with asthma (n=21) or without asthma (n=50) who were undergoing functional endoscopic sinus surgery in our tertiary center Otorhinolaryngology department. The diagnosis of NP was made according to the 2012 European position paper on rhinosinusitis and nasal polyps (11). Allergic rhinitis comorbidity in the NP patients was assessed with a skin prick test and by measuring serum-specific Ig-E levels. All patients had negative allergy findings. Control group patients had no illnesses or nasal symptoms. All of them underwent endoscopic nasal examination to rule out asymptomatic nasal polyps.

DNA samples of the 71 NP patients and 91 healthy controls were obtained from blood samples that were collected at the Biochemistry department of our tertiary center university hospital where the genotyping was performed.

Genotyping methodology: For determining the relationship between IL-1 β and TNF- α gene polymorphisms with NP, the purified DNA of 71 patients and 91 controls was analyzed by using a High Pure PCR Template Preparation Kit (Roche Diagnostics; GmbH, Mannheim, Germany). Polymorphism analysis was performed by using the same primers (IL-1 β -511 common, T, and C primers and TNF- α -308 common, A, and G primers), control primers (HGH, HGH II), and PCR conditions that were previously reported by McCarron et al. (12). SNPs were genotyped by

PCR using one reaction per allele for each SNP. All PCR reactions were performed in 10 μ l reaction volumes, and the final reagent concentrations were 10 \times reaction buffer (MBI Fermentas, Vilnius, Lithuania), 200 μ M each deoxynucleotide triphosphate (MBI Fermentas), 12% (w/v) sucrose (Merck, Darmstadt, Germany), 200 μ M cresol red (Aldrich, Steinheim, Germany), 1 μ M each specific/common primer (Metabion Int. AG, Martinsried, Germany), 0.2 μ M each internal control primer (Metabion Int. AG), 0.25 units of Taq DNA polymerase (MBI Fermentas), and 50-100 ng/ μ l DNA. MgCl₂ (Roche Diagnostics; GmbH, Mannheim, Germany) concentrations were optimized for each SNP as 2.75 mM per reaction for IL-1 β and 1.65 mM per reaction for TNF- α . PCR reactions were performed using a thermocycler (Uvigene, Biolab, UK) with the following conditions: 1 min at 96°C; 10 cycles of 96°C for 15 s, 65°C for 50 s, and 72°C for 40 s; 20 cycles of 96°C for 10 s and 60°C for 50 s; and 40 s at 72°C. PCR products were loaded directly onto 2% agarose gels (containing 0.5% mg/ml ethidium bromide), electrophoresed at 90 V for 40 minutes (Elite 300, Wealtec, Taiwan), and visualized by photography under UV transillumination (UVI PhotoV.99, Uvitec Ltd, UK). Genotypes were designated as CC, CT, and TT for IL-1 β and as AA, AG, and GG for TNF- α .

Statistical analysis

Genotype distribution was compatible with Hardy-Weinberg equilibrium. The Statistical Package for the Social Sciences for Windows (SPSS Inc.; version 11.5, IBM, Chicago, USA) statistical package was used for statistical analysis. The chi-square test was used for patient and control comparisons, and significance was assumed as $p < 0.05$. Logistic regression analysis was performed and the odds ratio (OR) was calculated when a genotype demonstrated a significantly different frequency than the others.

Results

The mean age of NP patients at presentation (50 males and 21 females) was 41 \pm 10 years (range 28-66 years). The mean age of the control group patients (73 males and 18 females) was 38 \pm 10 years (range 20-64 years). There was no statistically significant difference regarding age ($p = 0.097$) or sex ($p = 0.206$) between the study and control groups.

For TNF- α genotypes, four cases could not be determined for this polymorphism in the control group. It was likely that their DNA base sequences had differences at this point, and DNA sequencing analysis would be appropriate in these cases. The GG genotype in the TNF- α -308 region was a risk factor for NP development (OR:9.2, $p = 0.007$). There was no statistically significant difference between the TNF- α AA or GA genotypes regarding NP development ($p = 0.792$). Regarding allelic frequencies, the G allele was a risk factor for NP development in the TNF- α -308 promoter region (OR: 6.06, $p < 0.001$) (Table 1).

There was a statistically significant relationship between asthma in NP patients and TNF- α genotype in the -308 region ($p = 0.029$). Patients with asthma more commonly had the GG genotype in the TNF- α -308 region compared to non-asthmatic NP patients ($p = 0.000$) (Table 2).

Table 1. TNF-α-308 promoter region genotypes and allelic frequencies seen in patients with nasal polyps

		Patient (n=71)	Control (n=87)	p	Odds Ratio	95.0% CI	
						Lower	Upper
Genotypes	GG	60 (84.5%)	35 (40.2%)	0.007*	9.236	1.853	46.03
	AG	9 (12.7%)	38 (43.7%)	0.792	1.263	0.223	7.150
	AA	2 (2.8%)	14 (16.1%)		1		
Allelic frequencies	G	129 (90.8%)	108 (62.1%)	0.000*	6.064	3.174	11.585
	A	13 (9.2%)	66 (37.9%)		1		

*Statistically significant p-values
CI: confidence interval; G: guanine; A: adenosine

Table 2. Relationship between TNF-α-308 genotype and asthma in patients with nasal polyps

Genotype		Asthma			Total	p
		Absent	Present			
GG	Count	40	20	60	0.029	
	% within TNF-α	66.7%	33.3%	100.0%		
	% within Asthma	80.0%	95.2%	84.5%		
AG	Count	9	0	9		
	% within TNF-α	100.0%	.0%	100.0%		
	% within Asthma	18.0%	.0%	12.7%		
AA	Count	1	1	2		
	% within TNF-α	50.0%	50.0%	100.0%		
	% within Asthma	2.0%	4.8%	2.8%		
Total	Count	50	21	71		
	% within TNF-α	70.4%	29.6%	100.0%		
	% within Asthma	100.0%	100.0%	100.0%		

TNF: tumor necrosis factor; A: adenosine; G: guanine

There was no statistically significant relationship between asthma and allelic frequencies in the TNF-α-308 promoter region (p=0.253). Regarding IL-1β-511 promoter region genotypes, the CC genotype was a risk factor for NP development (OR:33.3, p=0.001). There was no statistically significant difference between TT and CT genotypes in the IL-1β-511 promoter region regarding NP development (p=0.519). There was no statistically significant difference between the allelic frequencies of T or C and NP development (p=0.166) (Table 3).

There was a statistically significant relationship between the IL-1β-511 promoter region genotypes and asthma in NP patients (p=0.021). The CT genotype was more common in NP patients having asthma compared to those without asthma (p=0.03) (Table 4). There was no statistically significant difference between allelic frequencies in the IL-1β-511 promoter region and asthma in the NP patients (p=0.641).

Table 3. IL-1β -511 promoter region genotypes and allelic frequencies seen in patients with nasal polyps

		Patient (n=71)	Control (n=91)	p	Odds Ratio	95.0% CI	
						Lower	Upper
Genotypes	TT	5 (7%)	1 (1.1%)	0.519	0.375	0.019	7.412
	CT	50 (70.4%)	89 (97.8%)	0.001*	0.030	0.004	0.241
	CC	16 (22.5%)	1 (1.1%)		1		
Allelic frequencies	T	60 (42.3%)	91 (%50)	0.166	0.732	0.470	1.138
	C	82 (57.7%)	91 (%50)		1		

*Statistically significant p-values
CI: confidence interval; C: cytosine; T: thymine; IL: Interleukin

Table 4. Relationship between IL-1β-511 genotype and asthma in patients with nasal polyps

Genotype		Asthma			Total	p
		Absent	Present			
CC	Count	14	2	16	0.021	
	% within IL-1β	87.5%	12.5%	100.0%		
	% within Asthma	28.0%	9.5%	22.5%		
CT	Count	31	19	50		
	% within IL-1β	62.0%	38.0%	100.0%		
	% within Asthma	62.0%	90.5%	70.4%		
TT	Count	5	0	5		
	% within IL-1β	100.0%	.0%	100.0%		
	% within Asthma	10.0%	.0%	7.0%		
Total	Count	50	21	71		
	% within IL-1β	70.4%	29.6%	100.0%		
	% within Asthma	100.0%	100.0%	100.0%		

IL: Interleukin; C: cytosine; T: thymine

Discussion

In this study, we found that there was a statistically significant increase in the expression of the TNF-α-308 GG and IL-1β-511 CC genotypes in the patients with NP. Comparison of allele frequencies showed that the G allele of the TNF-α-308 gene was higher in NP patients and in asthmatic patients with NP with a statistical significance. There was no statistically significant difference for having the T or the C allele of the IL-1β-511 gene polymorphism in NP patients and asthmatic patients with NP. In asthmatic patients with NP, there was a higher expression of the TNF-α-308 GG and IL-1β-511 CT genotypes.

Chronic rhinosinusitis with nasal polyps is an inflammatory disease of the paranasal sinuses and nasal mucosa that is diagnosed by bilateral endoscopically visualized polyps in the middle meatus with additional physical and/or radiological findings and symptoms presenting for longer than 12 weeks (11). It is seen more commonly in men and it has been associated with various medical conditions such as atopy, asthma, aspirin sensitivity, cys-

tic fibrosis, and ciliary dyskinesia (1, 9). Although NP is a widespread disease with a 1%-4% incidence, the exact underlying pathogenesis is not known (1). Mucosal inflammation with eosinophil predominance is presumably the most significant factor in its pathogenesis. The NP stroma contains various important molecules such as cytokines, chemokines, growth factors, and adhesion molecules that all take part in the cascade of reactions that take place during NP development (2). Although Th1 cytokines are also shown to be increased in the NP stroma, NP is characterized by a Th2-dominant cytokine profile. The increased eosinophil infiltration is related to Th2-triggered cytokines, including IL-4 and IL-5 (13).

TNF- α and IL-1 β are pro-inflammatory cytokines that are generated by several types of cells such as eosinophils, epithelial cells, and macrophages. They have synergistic effects in chronic inflammation in the presence of either Th1 or Th2 cytokines (13). Increased levels of TNF- α mRNA are observed in the NP tissue compared to the inferior turbinate, and there are also increases in the secretion of monocyte chemo-attractants from NP fibroblasts (14). Thus there is a vicious cycle between eosinophils and TNF- α production in the NP tissue. The major source of TNF- α in NP tissue is eosinophils (15). TNF- α upregulates vascular cell adhesion molecule-1 (VCAM-1) expression in fibroblasts, and this in turn increases the trans-migration of eosinophils to the inflammation site and leads to even more TNF- α production (16). Saji et al. (17) showed that the pro-inflammatory cytokines TNF- α and IL-1 β increased the secretion of RANTES from nasal polyp fibroblasts. RANTES is a chemo-attractant for monocytes, eosinophils, and memory T-cells, and it takes part in the cascade of reactions leading to the recruitment and trans-endothelial migration of cells into the inflammation site. Furthermore, when endothelial cells are exposed to these pro-inflammatory cytokines, they increase their expression of intercellular adhesion molecules and VCAM-1, which act as ligands for eosinophil integrins (18).

The TNFA gene locus is found in the highly polymorphic major histocompatibility III region on chromosome 6 (6p21.3). SNPs within the -308 promoter region of the TNF- α gene result in two allelic forms. The common form is called TNFA-1, and this has a G at this position. If G is substituted by A, the rare TNFA-2 allele appears (19). The results of previous studies investigating the allelic frequencies of TNF- α in the -308 promoter region on NP development are incompatible with each other. According to the results of Erbek et al. (7), AA polymorphism in the TNF- α -308 promoter region was higher in the control group. Bernstein et al. (9) found that the TNF- α A allele at position -308 increased the formation of NP almost two-fold compared to the control groups, whereas Erbek et al. (7), Mfunu Endam et al. (4), and Fajardo-Dolci et al. (10) found no statistically significant difference between TNF- α genotypes or allelic frequencies and NP development. Szabó et al. (20) found the A allele to be a risk factor for NP development only when acetylsalicylic acid sensitivity was present. In our results, the G allele frequency and the GG genotype were statistically significantly higher in NP patients compared to the control group. These different results in the studies can be explained by genetic variations and the different ethnicities of the study populations. TNF- α pro-

motor region allele frequencies can even change in study groups from the same region in the same disease (19). In the North America region, Randolph et al. (21) found that TNF- α gene promoter region allele frequency did not affect asthma formation, whereas Witte et al. (22) found the A allele to be a risk factor. The different results in the Turkish population (7, 8, and our study) can be explained by genetic variations in different regions of the country, patient selection criteria, and differences in study design. Moreover, the effect of allelic frequencies and genotypes of the TNF- α gene promoter region on the transcription of TNF- α is not clear. Greater production of TNF- α in the case of the GA genotype compared to the GG genotype has been reported in in-vitro studies, while other studies have found no correlation between genotype and transcription (23-27). In some gene reporter assays, the authors claim that the A allele influences TNF- α gene transcription, whereas others have found no such relationship (23, 28). There has been no published article investigating the role of allele frequency or genotype on TNF- α transcription in NP patients. The other important factor is that TNF- α expression is not only controlled by the TNFA gene locus, and the TNFB gene is another important molecular determinant of TNF- α levels (7). The extended linkage group around the TNFA locus might also be a causative factor for the genetic susceptibility for NP development (20).

Interleukin-1 is a crucial mediator in the pathogenesis of NP by activating T-cells and monocytes, inducing the expression of other cytokines, upregulating cellular adhesion molecules, taking part in the recruitment of eosinophils, and amplifying the reaction cascades of the inflammatory response (18, 29). IL-1 is found in two forms in humans IL-1 α and IL-1 β (both on chromosome 2) and they bind to the same receptor with different affinities (4, 29). The IL-1 β gene is located in the long arm of chromosome 2q14 (18). Besides its inflammatory effects in NP pathogenesis, IL-1 β is also responsible for glucocorticoid resistance in the NP tissue, making the treatment more complex (30). Like TNF- α , the results of the studies concerning the role of IL-1 β gene polymorphisms at the -511 promoter region in NP pathogenesis are also conflicting. Some authors have found no correlation between the IL-1 β genotype and NP development (4, 31), whereas the TT genotype (18) and the CC genotype (7) have been reported to be risk factors. Our results were similar to those of Erbek et al. (7), and we also found a higher CC genotype in NP patients and no relationship between allelic frequency (T or C) and NP formation. The IL-1 α GG genotype at the +4548 region has been demonstrated to be a genetic risk factor for NP development in asthmatic patients (29). According to our results, the CT genotype of the IL-1 β -511 region was a risk factor for asthma comorbidity in NP patients. The difference between previous published articles can also be attributed to different study design and ethnicity between study populations. In addition to IL-1 α and IL-1 β , the genes in the IL-1 complex code for a third protein called Interleukin-1 receptor antagonist (IL-1RA). This peptide is a confirmed competitive inhibitor of IL-1 β by binding to the IL-1 cell surface receptors, and it can down-regulate the pro-inflammatory responses elicited by IL-1 β . Linkage disequilibrium between the IL-1RA and IL-1 β genes might be the cause of NP development rather than IL-1 β alone (31).

Conclusion

The TNF- α GG genotype in the -308 position of the promoter region and the IL-1 β CC genotype in the -511 region might be genetic risk factors for NP development in the Turkish population. The effect of gene polymorphisms on the transcriptional expression of these pro-inflammatory cytokines should be investigated in further studies of NP patients.

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