Original Article / Çalışma - Araştırma

Non-steroidal anti-inflammatory drug-activated gene-1 expression levels in nasal polyposis

Nazal polipoziste nonsteroid antienflamatuvar ilaç ile active gen-1 ekspresyon düzeyleri

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Objectives: This study aims to investigate the possible role of non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1) in nasal polyp development.

Patients and Methods: Twenty-one patients (15 males, 6 females; mean age 44.3 years; range 16 to 65 years) who underwent endoscopic sinus surgery for nasal polyposis (NP) were included in the study. Inferior turbinate mucosa samples were taken in addition to the polyp tissue which was already removed during routine procedure. The NAG-1 gene messenger ribonucleic acid (mRNA) expression levels of the polyp tissue and healthy turbinate mucosa were examined by real-time polymerase chain reaction (PCR). Patients were divided into two groups based on the presence or absence of comorbid asthma.

Results: The NAG-1 gene expression of the polyp tissue was 1,089 fold higher, compared to the healthy nasal mucosa (p=0.757). The NAG-1 mRNA levels were 2.13 times decreased in the patients with comorbid asthma (p=0.275). There was no statistically significant difference between the groups.

Conclusion: With the findings of this study NAG-1 gene may play a role in nasal polyp development in the presence of comorbid asthma.

Key Words: Asthma; inflammation; nasal polyposis; non-steroidal anti-inflammatory drug-activated gene-1.

Amaç: Bu çalışmada nonsteroid antienflamatuvar ilaç ile aktive gen-1'in (NAG-1) nazal polip gelişimindeki muhtemel rolü araştırıldı.

Hastalar ve Yöntemler: Nazal polipozis nedeniyle endoskopik sinüs cerrahisi yapılan 21 hasta (15 erkek, 6 kadın; ort. yaş: 44.3 yıl; dağılım 16-65 yıl) çalışmaya dahil edildi. Rutin işlem sırasında alınan polip dokunun yanı sıra, alt konka mukozasından da örnek alındı. Polip dokusunda sağlıklı konka mukozasında NAG-1 gen mesajcı ribonükleik asit (mRNA) ekspresyon düzeyleri gerçek zamanlı polimeraz zincir reaksiyonu (PZR) ile değerlendirildi. Hastalar eşlik eden astım olup olmadığına göre iki gruba ayrıldı.

Bulgular: Polip dokusunun NAG-1 gen ekspresyonu, sağlıklı burun mukozasına kıyasla, 1.089 kat daha fazlaydı (p=0.757). NAG-1 mRNA düzeyleri, eşlik eden astımı olan hastalarda 2.13 kat azalmıştı (p=0.275). Gruplar arasında istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: Bu çalışmanın sonuçlarına göre NAG-1 geninin astım komorbiditesi olan hastalarda nazal polip gelişiminde rolü olabilir.

Anahtar Sözcükler: Astım; enflamasyon; nazal polip; nonsteroid antienflamatuvar ilaç ile aktive gen-1.

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Nasal polyposis (NP) is the chronic inflammatory disease of the mucosa covering the nasal cavity and paranasal sinuses. It is usually characterized by benign mucosal protrusions (polyps) originating from the middle meatus and anterior ethmoidal cells that grow down between the lateral nasal wall and middle turbinate and result in clinical symptoms such as nasal obstruction, nasal/postnasal drainage, facial fullness and hyposmia/anosmia.[1] In the general population, the prevalence varies between 1-4% and often affects adults.[2] Despite developments in nasal physiology, immunohistochemistry, histopathology and microbiology, NP pathophysiology is not yet clearly understood and commonly thought to be multifactorial. Allergy, fungal and bacterial infections and biofilms, various chemical mediators such as interleukin (IL-5), Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), matrix metalloproteinase (MMP), tumor necrosis factor (TNF), nitric oxide (NO), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), hypoxia due to osteomeatal complex obstruction, cyclooxygenase (COX) metabolism and some bacterial superantigens like staphylococcal enterotoxins all play a role in NP pathogenesis.[3,4] Chronic inflammation and mucosal edema is considered to be the most important factor in nasal polyp development. Genetic basis is also suspected because of NP associated diseases like ASA triad (also known as Samter's triad; nasal polyposis, asthma, aspirin sensitivity) and cystic fibrosis. Recurrence despite adequate medical and surgical treatment also point to genetic predisposition.[3,5]

Nonsteroidal anti-inflammatory drug activated gene-1 (NAG-1) which is a divergent member of TGF-β gene superfamily was first isolated in human colorectal cancer cells.[6,7] It has anti-inflammatory, antitumor and pro-apoptotic properties.[8,9] In this study we determined to ascertain the potential effect of NAG-1 gene in nasal polyp development. Therefore we decided to compare NAG-1 gene expression levels in polyp tissue and normal nasal mucosa of the same patients with NP.

PATIENTS AND METHODS

This study was conducted in Gazi University Otorhinolaryngology Department between July 2010 and March 2011 after obtaining University Ethics Committee approval. Twenty-one patients (15 males, 6 females; mean age 44.3 years; range 16 to 65 years) who were undergoing endoscopic sinus surgery for nasal polyposis were consecutively enrolled in the study after securing informed consent. All the patients had been given local and systemic steroid treatment previously. They were examined for comorbidities such as asthma, aspirin sensitivity and allergic rhinitis with the appropriate diagnostic tools. The asthma and allergic rhinitis diagnosis were confirmed with pulmonary function test and skin prick test. The exclusion criteria from the study included ASA triad, cystic fibrosis and age below 15-years. In the operation a healthy nasal mucosa sample was taken from the inferior turbinate of the same patients in addition to the polyp tissue that was already removed during the routine procedure, to compare NAG-1 gene expression levels.

Ribonucleic acid (RNA) isolation and complementary deoxiribonucleid acid (cDNA) synthesis

The nasal polyp and control samples were immediately frozen in liquid nitrogen and stored at -80 °C until the RNA extraction procedure was performed. About 100 mg of the sample was homogenized using an IKA T_{10} Basic Ultra-Turrax homogenizer (IKA-Werke, Staufen, Germany). Total RNA isolation from tissue homogenate was performed by phenol-guanidine thiocyanate extraction using RNAzol® RT reagent (Molecular Research Center, Inc. Cincinnati, OH) according to the manufacturer's instructions and its concentration determined by measuring the absorbance at 260 and 280 nm using the NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Total RNA (1 mg) was reverse-transcribed to cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany) in a 20 mL reaction mixture. Random hexamers were used to prime the cDNA synthesis.

Quantitative Real-time polymerase chain reaction (PCR) and statistical analysis

Real-time PCR was performed using a Light Cycler 480 II System (Roche Diagnostics GmbH, Mannheim, Germany). To quantify cDNA we performed real-time quantitative PCR using LightCycler 480 Probe Master mix and hydrolysis probes (TaqMan probe, Roche Diagnostics GmbH, Mannheim, Germany). Primer pairs and specific

Table 1. Gene-specific primer sequences and probe numbers for real-time polymerase chain reaction

Gene	Forward primer	Reverse primer	Universal probe library no.
GAPDH	5′-AGCCACATCGCTCAGACAC-3′	5′-GCCCAATACGACCAAATCC-3′	Human #60
NAG-1	5′-CCGGATACTCACGCCAGA-3′	5′-AGAGATACGCAGGTGCAGGT-3′	Human #28

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NAG-1: Nonsteroidal anti-inflammatory drug activated gene-1.

probes were selected using the on-line Universal Probe Library Assay Design Center.[10] Table 1 shows primers and probes used in real-time PCR for each gene. Real-time PCR was performed according to the following conditions: activation of Taq DNA polymerase and DNA denaturation at 95 °C for 10 minutes, followed by 50 amplification cycles for 10 seconds at 95 °C and 20 seconds at 60 °C and finally a cooling step to 40 °C. For each sample, the levels of the target gene transcripts were normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Expression of the target genes' messenger RNA (mRNA) relative to that of the housekeeping gene mRNA was calculated using the relative expression software tool (REST 2009, V2.0.13), and statistical significance was determined using the pair-wise fixed reallocation randomization test.[11] P<0.05 was considered statistically significant.

RESULTS

Demographic data of the patients are summarized in Table 2. There were six patients (28.5%) with asthma, one patient with aspirin sensitivity and two patients with allergic rhinitis. Five patients had previously undergone endoscopic sinus surgery for nasal polyposis. We observed heterogenic gene expression levels among the patients meaning that some of them showed increased, whereas others showed decreased levels of NAG-1 mRNA. On average, the NAG-1 gene was 1.089 times more expressed in polyp tissue compared to healthy nasal mucosa (Table 3) but there was no statistically significant difference (p=0.757).

Table 2. Demographics of the study group

	Gender		Age	
	n	%	Mean	Range
Male	15	71.4	42.2	16-65
Female	6	28.5	48.8	28-63
Total	21	100.0	44.3	16-65

The statistical analyses were reevaluated after dividing the patients into two subgroups based on having comorbid asthma or not. There was a 2.13 times decrease and 1.59 times increase of NAG-1 mRNA expression in patients with and without comorbid asthma respectively (Figure 1). The difference in the gene expression level were not statistically significant in any subgroup (p=0.275 and p=0.055 respectively).

DISCUSSION

The NAG-1 gene which is also named as macrophage inhibitory cytokine-1 (MIC-1), placental transformation growth factor-β (PTGF-β), prostate-derived factor (PDF), growth differentiation factor 15 (GDF15) and placental bone morphogenetic protein (PLAB) was first shown to exhibit anti-tumorigenic and pro-apoptotic effect on colon carcinoma cells. It has been shown that cancer patients develop up-regulation in NAG-1 gene expression in response to nonsteroidal anti-inflammatory drugs (NSAIDs) such as COX inhibitors.[12]

There are many additional studies in the literature regarding the possible relationship between the NAG-1 gene and cancer development.[13-16] With respect to these oncologic studies regarding the NAG-1 gene, we were actually not able to consider a directly relation to NP etiology. However the relationship between inflammation and cancer development is also suspected.[17,18] Chronic inflammation is known to result in DNA damage, unlimited replication and inhibition of apoptosis which may eventually result in cancer development.[19] There are also some authors who put forward that anticarcinogenic effects of NAG-1 gene are related to anti-inflammatory properties of the gene.[20,21]

Additionally there are also some other non-oncologic studies in the literature regarding the NAG-1 gene. Bootcov et al.[8] showed that NAG-1 gene is inducted with TNF-α and IL-1 which is secreted from activated macrophages. Also, NAG-1 gene expression in macrophages resulted

Table 3. The expression levels of NAG-1 mRNA relative to GAPDH mRNA in NP tissue compared with control

Gene	Tip	Reaction efficiency	Expression	Standard deviation	95% CI	*p*
GAPDH	REF	1	1			
NAG-1	TRG	1	1,089	0.401-2.972	0.069-8.056	0.757

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NAG-1: Nonsteroidal anti-inflammatory drug activated gene-1; REF: Reference gene; TRG: Target gene.

in inhibition of TNF-α secretion, so that it has shown anti-inflammatory effect on macrophages. Taoka et al.[22] shown that NAG-1 gene down-regulation was correlated to inflammatory changes in prostate tissue.

There were two studies in the literature regarding the expression of NAG-1 gene in the sinonasal region to date. Kim et al.[23] first showed the expression of NAG-1 gene in the nasal mucosa. They compared the gene expression in different compartments of the nasal mucosa and found out that NAG-1 gene was more expressed in highly differentiated apical ciliated cells compared to basal and goblet cells. Kim et al.[24] studied the antineoplastic effect of the COX inhibitor indomethacin on sinonasal cancer cell line and they demonstrated the antineoplastic and pro-apoptotic effect of indomethacin against sinonasal cancer cells via induction of NAG-1 gene.[24] According to these studies it may be postulated that NAG-1 gene might be responsible for differentiation and apoptosis of nasal epithelial cells.

In this study, we examined NAG-1 gene expression in nasal polyp tissue with real-time PCR for the first time. It is also the second published study showing NAG-1 gene expression in nasal mucosa. We postulated that NAG-1 should be down-regulated in polyp tissue compared to nasal mucosa but we did not see any significant difference in gene expression levels between polyp tissue and healthy nasal mucosa. There were heterogenic expression levels among the patients meaning that the NAG-1 gene was up-regulated in some cases and down-regulated in others. Thus we subdivided the patients based on presence or absence of asthma comorbidity as mentioned in the results section.

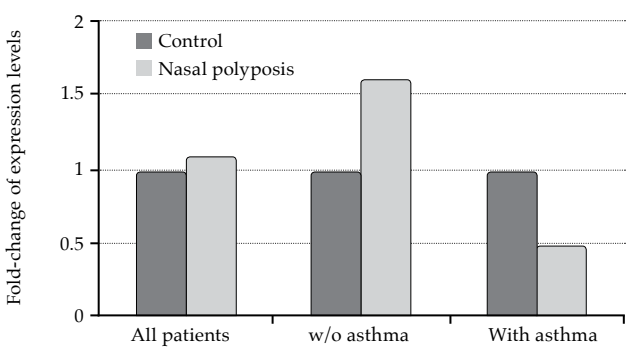

Figure 1. *The expression levels of NAG-1 gene in the polyp tissue and healthy nasal mucosa (control) of the individuals with/without comorbid asthma.*

It was shown that NAG-1 mRNA expression was lower in patients with asthma comorbidity leading us to wonder whether the decreased inhibitory effect of the NAG-1 gene on macrophages and other unknown targets resulted in chronic inflammation and eventually manifested with symptoms of nasal polyposis and asthma; however the difference was not significant enough to propose that.

Even though the differences were not statistically significant, it was shown that gene expression was increased in patients without comorbid asthma and decreased in patients with comorbid asthma. This may be attributed to the different pathophysiologic mechanisms that play a role in such NP subgroups as aspirin sensitivity, asthma, cystic fibrosis or isolated NP.

It is clearly known that asthma and nasal polyposis are related diseases, but there are few studies in the literature that examine this relationship on the genetic or protein level. The present studies are commonly conducted on ASA triad but there are a huge number of patients suffering from asthma and nasal polyposis who are insensitive to aspirin. Bachert et al.[25] have shown that patients with high staphylococcal enterotoxins immunoglobulin E (IgE) antibody and IL-5 levels in polyp tissue have higher asthma comorbidity ratio. The lower NAG-1 mRNA expression levels in polyp tissue of the asthma subgroup shown in this study, with contribution of the other studies in the future, should be taken in account in the NP-asthma comorbidity pathophysiology.

In this study, inferior turbinate mucosa of the same patient was used for control to examine the possible relationship between NP and NAG-1 expression. Platt et al.[26] used a similar study design; examining gene expression in five distinct sinonasal anatomic subsites of the nasal polyposis and control groups. They found regional alterations in gene expression not only between groups but also in the different anatomic subsites of the same individuals. It is well known that polyp tissue develops from middle meatal and paranasal sinus mucosa but not from inferior turbinate mucosa. This condition might be related to differences in genetic expression levels in different sinonasal anatomic subsites and the best way to examine this possible variation is to compare the pathologic material and neighboring healthy nasal mucosa of the same individual.

In conclusion, NAG-1 mRNA expression was first shown in nasal polyp tissue with real-time PCR in our study. When we compare the gene expression in normal nasal mucosa and polyp tissue of the two subgroups which have or lack asthma comorbidity; it is seen that NAG-1 expression was decreased and increased respectively. These findings have again shown that not all nasal polyposis cases can be explained with a single etiopathogenetic mechanism, and also made us consider that asthma and NP association may be related to NAG-1 gene suppression. It is apparent that it is not yet possible to conclude a relation between NP and NAG-1 gene with these findings. Further genetic studies including the protein level with more subjects and patient subgroups such as cystic fibrosis, aspirin sensitivity and ASA triad are needed to determine the possible relationship with NAG-1 gene and NP.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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