Is There an Association Between Nasal Polyposis and ADAMTS Genes Expressions?

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ABSTRACT

Objective: Nasal polyposis (NP) is an inflammatory chronic disease in which polyps are located in the nose or paranasal sinuses. A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) genes have roles in vascular biology, inflammation, tissue morphogenesis, and pathophysiological remodeling. Therefore, some members of the ADAMTS gene family may contribute to pathogenesis of NPs. This study aimed to detect the potential relation between NP and the expression levels of ADAMTS 5, 8, and 9 genes.

Materials and Methods: This study consisted of nasal polyp tissues from 34 patients in whom nasal polyps had been diagnosed clinically, and healthy nasal mucosal tissues from 14 controls. RNA was isolated from the nasal polyps and normal nasal mucosal tissue in each subject. The expression levels of ADAMTS 5, 8, and 9 genes in the patients and controls were detected by quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) method.

Results: The expression levels of ADAMTS 5 and 9 genes were significantly decreased in NP tissues. In contrast, the expression levels of ADAMTS 8 genes were also decreased in NP tissues, but they were not significantly different from those in the normal nasal tissues.

Conclusion: An association was detected between the expression levels of ADAMTS genes and NP. ADAMTS 5 and 9 genes may have an effect on the formation of NP.

Keywords: Nasal polyps, ADAMTS-5, ADAMTS-8, ADAMTS-9

Introduction

Nasal polyposis (NP) is an inflammatory chronic disease in which polyps are located in the nose or paranasal sinuses. In NP, the edematous mucosal masses prolapse into the nasal cavity and result in nasal obstruction, increased nasal secretion, anosmia, headaches, and disturbances in life quality [1]. NP is a multifactorial condition, which is often associated with allergy, asthma, cystic fibrosis, infection, and aspirin intolerance [2]. In different populations, the prevalence of NP is about 1–4% [2, 3]. Infiltration of inflammatory cells, abnormal angiogenesis, remarkable edema, submucosal fibrosis, diminishing of mucous glands, and mucosal epithelial hyperplasia are the features of NP. The chronic inflammation in NP is characterized by inflammatory cytokines and chemokines [4]. Edema and polyp growth occur with an increase in capillary and basilar membrane permeability. However, pathogenic mechanisms underlying NP are not defined clearly [5]. Blood vessels in nasal polyps have been reported as immature and have abnormal innervation. The formation of new blood vessels and angiogenesis may contribute to NP [6]. In some studies, it is concluded that one of the main angiogenic factors, vascular endothelial growth factor (VEGF) inducing edema and angiogenesis, was involved in the pathogenesis of nasal polyps [7-9].

A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) are extracellular zinc metalloproteases. It is known that ADAMTSs cleave various substrates in the extracellular matrix. They are associated with extracellular matrix turnover, blood coagulation, ovulation, and melanoblast development. ADAMTSs also take part in cancer, angiogenesis, atherosclerosis, arthritis, and wound healing. ADAMTSs 5, 8, and 9 have antiangiogenic functions like most of the other ADAMTSs [10]. Therefore, they contribute to nasal polyp formation owing to their func-

tions in angiogenesis. To the best of our knowledge, there is no information on ADAMTS gene expression in nasal polyps.

Thus, this study aimed to investigate the expression levels of ADAMTS 5, 8, and 9 genes in NP to shed light on the etiopathogenesis of NP.

Materials and Methods

Subjects and Surgery

This study consisted of NP tissue (*n*=34) and healthy tissue (*n*=14) from subjects aged 35–62 years. Written informed consents of the subjects and decision of the ethical committee of Medical Faculty of Ataturk University (decision number; 2013-12) were available. The NP group was chosen from patients with chronic rhinosinusitis who attended an otorhinolaryngology clinic. The control group comprised subjects without any symptoms or with a history of NP. Nasal mucosal tissue removed during turbinoplasty was used for control. All the NP patients were first examined endoscopically and diagnosed clinically. The patients who approved and adopted surgical therapy were operated. Patients with a confirmed diagnosis of NP were included in the study after a histopathological examination. Patients had not used steroids (systemic or topical), nonsteroidal anti-inflammatory drugs, or antihistamines, for four weeks before the biopsy.

The exclusion criteria were: mucoceles, antrochoanal polyps, benign–malignant tumors, fungal sinusitis, cystic fibrosis, ciliary dysfunction, immune deficiency, previous respiratory tract infection or treatment with systemic and topical corticosteroids, antibiotics and antihistamines for four weeks before the biopsy, previous sinus surgery, allergic rhinitis, asthma, or aspirin sensitivity.

RNA Isolation and Gene Expression Studies

RNA isolation was performed on 1´1 cm^2 tissue samples (approximately 20 mg). For RNA isolation, a High Pure RNA Tissue Kit (Roche Diagnostics, GmbH, Mannheim, Germany) was used. The purity and quantity of total RNA were measured by spectrophotometry (NanoDrop: Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA (cDNA) was synthesized using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, GmbH, Mannheim, Germany). A polymerase chain reaction (PCR) mixture was incubated in a GeneAmp® PCR System 9700 (Applied Biosystems, NY, USA) at 55 °C for 30 min and at 85 °C for 5 min. The expression of the target genes, ADAMTSs 5, 8, and 9, and the housekeeping gene (***beta-actin***) was analyzed with LightCycler® FastStart DNA Master^{PLUS} HybProbe (Roche Diagnostics, GmbH, Mannheim, Germany) protocols. Relative expression values of ADAMTS***/beta-actin*** and melting curve analysis were performed by LightCycler 480 Real-Time PCR (Roche Diagnostics, GmbH, Mannheim, Germany).

Statistical Analysis

The one-sample ***t***-test was conducted to determine differences in the expression levels between the controls and patients. All statistical calculations were performed using the Statistical Package for Social Sciences software version 20.0 (IBM SPSS Corp.; Armonk, NY, USA) statistical program. *p*-values ≤ 0.05 were considered significant (Table 1).

Table 1. The expression levels of the ADAMTS genes in nasal polyposis and normal tissues

Genes	Nasal polyposis (mean±SD)	Control tissues (mean±SD)	p values
ADAMTS 5	0.00017794±0.00026952	0.00023587±0.00018403*	0.001
ADAMTS 8	0.00010001±0.00002871	0.00012070±0.00000750	0.128
ADAMTS 9	0.00100471±0.00250498	0.00192543±0.00291139*	0.028

*≤0.05.
ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs. SD: standard deviation.

Main Points

- Infiltration of inflammatory cells, abnormal angiogenesis, remarkable edema, and mucosal epithelial hyperplasia are the features of nasal polyps.
- Main angiogenic factor, vascular endothelial growth factor (VEGF) inducing edema and angiogenesis, was involved in the pathogenesis of nasal polyps.
- ADAMTS 5, 8, and 9 have antiangiogenic functions and associated with inflammatory cytokines.
- In this study, reduced expression levels of ADAMTS 5, 8, and 9 genes was detected.
- The reduced expression levels of these genes might be associated with upregulation of VEGF-induced angiogenesis and contribute to the pathogenesis of nasal polyps.

Results

Beta-actin gene (a housekeeping gene used as an internal control for gene expression normalization) expression was determined in all the patient and control tissues. The expression levels of ADAMTS 5 and 9 genes were significantly decreased in NP tissues when compared with those in the normal tissues ($p \leq 0.05$) (Table 1). The expression of ADAMTS 8 gene was decreased, but it was not a significant decrease ($p > 0.05$).

Discussion

Nasal polyps are noncancerous swellings that grow inside the nasal cavity or sinuses. NP is an inflammatory chronic disease characterized by polyps in the nose or paranasal sinuses. NP can originate from different etiological factors. However, persistent inflammation is the most frequent cause [11]. There are several opinions about the mechanisms underlying NP, with some studies pointing to roles for chronic infection, aspirin intolerance, alterations in aerodynamics and nasal airflow, epithelial destruction, epithelial cell defects associated with cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations, various allergic agents, and sodium absorption defects [11]. Local mucosal and environmental factors are also thought to explain NP, in addition to familial factors, with a predisposition to NP thought to an inherited trait [12]. Various key inflammatory cytokines and chemokines are secreted in the process of NP. Increased immune cells, such as lymphocytes, mast cells, and eosinophils act in the inflammatory process of NP [4]. Another mechanism of pathogenesis for the chronic rhinosinusitis with NP is the defect in the sinonasal epithelial barrier. Reduction or improper placement of tight junction proteins and increased epithelial permeability leads to the defect. IL-4, IL-13, and oncostatin M may contribute to disruption of the sinonasal epithelial barrier. The ***in vitro*** combination of IL-4 or IL-13 and tumor necrosis factor (TNF) increases the important chemokine CCL11 expressed in NP. [4]. Some studies have shown that angiogenesis is evident in NP and contribute to the formation of NP [5, 6]. It is reported that VEGF expression was upregulated in nasal polyps [7-9].

VEGF has a critical role in embryonic vasculogenesis and/or angiogenesis, vascular permeability, endothelial cell proliferation, and tumor angiogenesis. VEGF is associated with tissue homeostasis mechanisms, such as proliferation and apoptosis, and might be involved in nasal polyp formation (7).

Several gene expression studies report that various genes, such as SOCS3, SOCS1, GILZ, MET, PPP1R9B, PIP, and AZGP1, are involved in the pathogenesis of NP [13-15].

Proteases contribute to remodeling of the vascular basal membrane in the angiogenesis process. It is reported that proteases are nega-

tive regulators of angiogenesis [16]. ADAMTS proteases have similar domains with some of the antiangiogenic molecules [16].

It is demonstrated that ADAMTSs 5 and 8 are endogenous angiogenesis inhibitors. ADAMTS 8 inhibits endothelial cell proliferation, reversibly. ADAMTS 9 has an antiangiogenic mechanism by proteolysis. Increased ADAMTS 9 correlates with reduced proangiogenic factors, such as matrix metalloproteinase-9 and VEGF [10]. ADAMTS 9 has 15 potentially antiangiogenic domains. ADAMTS 9, expressed by microvascular endothelial cells, has a cell-autonomously acting antiangiogenic function [16].

In this study, we investigated the expression of ADAMTS 5, 8, and 9 genes in NP. We detected reduced expression levels of the three genes in NP. However, only the expressions of ADAMTS 5 and 9 genes were significantly diminished. The reduced expression levels of these genes might be associated with upregulation of VEGF-induced angiogenesis. It is reported that ADAMTS 5 and 9 genes are associated with various cytokines, which have functions in inflammation and angiogenesis. These cytokines are IL-1, IL-6, TNFα, and leptin for ADAMTS 5 and IL-1, TNFα, leptin, and oncostatin M for ADAMTS 9 [17]. Therefore, ADAMTS 5 and 9 genes might be concerning in NP pathogenesis more than ADAMTS 8. There appear to be no other reports of the association between ADAMTS and NP in the literature. ADAMTSs 5 and 9 may contribute to pathogenesis of NP.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ataturk University (Approval Number: 2013-12).

Informed Consent: Informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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