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Evaluation of patients with nasal polyps about the possible association of desmosomal junctions, RORA and PDE4D gene

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Abstract. – OBJECTIVES: Nasal polyposis is chronic inflammatory disease of the nasal mucosa of the nose and sinuses, often associated with chronic non-allergic rhinitis, aspirin intolerance and non-allergic asthma. The etiology of nasal polyposis is unknown. Multiple factors contribute to the development of nasal polyps including genetic predisposition.

PATIENTS AND METHODS: This study was conducted on patients applied due to nasal polyps. Blood samples were collected peripheral vein and stored at 4°C until analysis for DNA extraction. Genomic DNA was extracted from peripheral blood by a standard method, samples were studied in real time PCR. All patients were evaluated about the possible association of DSG1 (rs7236477-G, 96 rxn), DSG3 (rs1941184-C, 96 rxn), PDE4D (rs1588265) and RORA (rs11071559) gene.

RESULTS: 32 patients (17 male, 15 female) with nasal polyposis were included to the study. The mean age was 34.9 ± 17.7 years, ranging between 18 and 55 years. Control group was consisted with 50 healthy volunteers without a history of nasal polyp. DSG1, DSG3 and RORA values of the study group were not statistically different from control group ($p > 0.05$). PDE4D values of the study group were significantly different from control group ($p < 0.05$).

CONCLUSIONS: Multiple factors contribute to the pathogenesis of nasal polyps including genetic predisposition. The PDE4D family has gained interest in the complex pathogenesis of nasal polyposis. This is likely linked to the mucosal inflammatory response.

Key Words:
Nasal polyposis, Desmosomal junctions, RORA, PDE4D, Chronic sinusitis.

Introduction

Nasal polyps are described benign edematous masses, as a common chronic inflammatory disease of the nasal mucosa that originate from the mucosal linings of the paranasal cavity usually in the osteomeatal complex[1]. The presenting symptoms include nasal obstruction, epistaxis, mouth breathing, hyposmia, anosmia, watery rhinorrhea, snoring, halitosis, nasal pruritus, headache, dyspnea, dysphagia, dysphonia and leading to acute or chronic sinusitis[2].

The etiopathogenesis of nasal polyposis is currently unknown. Multiple factors contribute to the development of nasal polyps including genetic predisposition, neoplastic and neurovascular factors, mucosal reactions including allergic inflammation and infection, inflammatory mediators released from mucus glands and alterations in stromal connective tissue, and anatomic abnormalities in the ethmoid sinuses[3,4]. The formation has been linked to proliferation of the epithelial layer, focal fibrosis, cellular infiltration of the stromal layer, stromal edema, eosinophilic infiltration and remodeling of epithelium, glands and connective tissues, but has not been linked to a change in the angiogenesis of the architecture of the capillary bed[5,6].

It have been shown that disruption of the tight junction and the adherens junction are play important roles in the pathogenesis of several mucosal inflammatory conditions such as allergic inflammation, inflammatory bowel disease, and a variety of infections[7]. However, increasing evidence of the role of barrier forming tight junctions in mucosal inflammation, very little is known about the contribution of desmosomes to nasal epithelial biology and polyp formation. Desmosomes are composed of desmosomal cadherins termed desmogleins (DSG) and desmocollins (DSC) and important elements of intercellular junctions[8]. Cyclic nucleotides are important second messengers that regulate and mediate a number of cellular responses to extracellular signals, such as hormones, light, and neurotransmit-

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ters. Cyclic nucleotide phosphodiesterases (PDEs) regulate the cellular concentrations of cyclic nucleotides and, thereby, play a role in signal transduction. PDE enzymes, ubiquitously distributed in mammalian tissues, are essential to control numerous physiological processes[9]. PDE4D is a class IV cAMP-specific PDE. Retinoic acid-related orphan receptor-alpha (RORA) has been implicated in the pathology of bone growth, angiogenesis, cellular metabolism and a mediator in the immune and lipid metabolism pathways[10].

The aim of this study was to assess the possible association of DSG1 (rs7236477-G, 96 rxn), DSG3 (rs1941184-C, 96 rxn), PDE4D (rs1588265) and RORA (rs11071559) gene in the patients with nasal polyps.

Patients and Methods

This prospective study was conducted on patients applied due to nasal polyps to our Ear Nose and Throat Clinics at Abant Izzet Baysal University Hospital, Bolu, Turkey. The study was conducted with the approval of the Ethics Committee of the Medical Faculty of Abant Izzet Baysal University Bolu, Turkey. The diagnosis of chronic rhinosinusitis with nasal polyposis was based on history, physical examination with nasal endoscopic evaluation and sinus computed tomography (CT) scan. All patients undergoing first surgical removal of unilateral or bilateral nasal polyps were included. The control group was consist of patient without nasal polyps. Blood samples were collected peripheral vein and stored at 4°C until analysis for DNA extraction. Genomic DNA was extracted from peripheral blood by a standard method (High Pure PCR Template Preparation Kit-Roche). After genomic DNA obtained, samples were studied in real time PCR (lightCycler480 II), (Roche Diagnostics, Mannheim, Germany) with Light SNIP (single nucleotide polymorphsm) PDE4D, Light SNIP DSG1, Light SNIP DSG3 and Light SNIP RORA pob kits (TIB MOLBIOL GmbH, Berlin, Germany).

Mixture for PCR: We prepared a mixture with 14.4 µl PCR grade water (H_2O), 1.0 µl Reagent mix, 2.0 µl FastStart DNA master Hybprobe (Roche Diagnostics, Mannheim, Germany). 1.6 µl $MgCI_2$ (25 mM) and 5 µl of DNA sample. Mixture was delivered to the wells on microplates up to the number of patients. Sample was assessed in Light Cycler 480-II devices after mixture in microplates closed in gelatin and centrifuged.

Statistical Analysis

Analyses were performed with Epi Info version 3.5.3. Hardy-Weinberg equilibrium and the absence of LD were evaluated using the allele procedure of Epi Info version 3.5.3. All statistical tests were two sided and a nominal *p* value low of 0.05 was considered statistically significant.

Results

32 patients (17 male, 15 female) were included to the study. The mean age was 34.9 ± 17.7 years, ranging between 18 and 55 years. Control group was consisted with 50 healthy voulnteers without a history of nasal polyp. Study analyses performed by evaluation of the peaks on the basis of Tm value (melting peaks). Each sample evaluated as either wild type, heterozygous type or homozygous type.

Evaluation of the Peaks

For RORA (rs11071559): 52.99 allel Timin (T) and 60.97 for allel Cytosin (C). For DSG1 (rs7236477): 53.50 Allel Adenin (A) and 60. 77 for allel Guanin (G). For DSG3 (rs1941184): 54. 20 allel G and 60.06 for allel T. For PDE4D (rs1588265): 55. 32 allel G and 60.87 for allel A RORA polymorphism of the control group was as follows; 1 subject had TT genotype, 32 subjects had CC genotype and 19 subjects had CT genotype. DSG1 polymorphism was as follows in control group; 45 subjects had AA, 1 subject had GG and 4 subjects had AG genotypes. DSG3 polymorphism of the control group was as follows; 24 subjects had TT, 3 subjects had GG and 23 subjects had TG genotypes. PDE4D polymorphism of the control group was as follows; 22 subjects had AA, 11 subjects had GG and 17 subjects had AG genotypes. RORA polymorphism of the study group was as follows; 24 patients had CC, 1 patient had T and 7 patients had CT genotypes. DSG1 polymorphism of the study group was as follows; 30 patients had AA, 1 patient had G and 1 patient had AG genotypes. DSG3 polymorphism of the study group was as follows; 17 patients had TT, 3 patients had G and 12 patients had TG genotypes. PDE4 polymorphism of the study group was as follows; 15 patients had AA, 2 patients had GG and 15 patients had AG genotypes.

DSG1, DSG3 and RORA values of the study group were not statistically different from control group ($p > 0.05$). However, PDE4D values of the study group were significantly different from control group ($p < 0.05$).

Discussion

Many reports have led to proposed hypotheses as to the origination of nasal polyposis. From these reports the polyposis may result from prolapse of the lamina propria, epithelial damage, gland formation, epithelization of the prolapse, elongation and enlargement due to gravity, and changes in the epithelium and stroma. Despite these studies, it is still not clear what sequence of events in nasal epithelium occurs resulting in the rupture of the lamina propria, inflammation, edema, and polyps formation[9,11].

In the patient with allergic rhinitis, nasal mucosa, structure, protein expression and function of the tight junctions are well maintained[12]. DSG1 and DSG3 belong to the desmosomal cadherins and act as intercellular adhesion molecules that connect the epidermal keratinocytes[13,14]. The DSG3 is expressed in basal and immediate suprabasal layers of skin and across the entire stratified squamous epithelium of oral mucosa[15]. However, increasing evidence suggests that the role of DSG3 may involve more than just cell-cell adhesion. The expression of the desmosomal protein DSG2 and DSG3 is greatly decreased in human nasal polyps in comparison with expression observed in normal nasal mucosa[9]. Jang et al[15] examined the expression of the tight junction proteins and adherens junction proteins in nasal polyposis. They reported that the tight junction protein ZO-1 was down-regulate due to epithelial dedifferentiation, while the adherens junction protein E-cadherin was up-regulated in a fashion that correlated with increasing severity of epithelial remodeling. But Zuckerman et al[8] was not show a significant difference in tight junctional proteins or adherens junctional protein expression in nasal polyps compared with that observed in normal nasal mucosa. A analysis of desmosomal junctions comparing asthmatic and nonasthmatic polyps by Shahana at al[17] that they said there was a reduced relative length of columnar desmosomes implying that asthmatic patients and allergic patients may have desmosomal weakening. We had a relatively small sample size and we did not see a appreciable statically differences between the control group and patient with nasal polyps about DSG1 (rs7236477-G, 96 rxn), DSG3 (rs1941184-C, 96 rxn).

PDE enzymes, ubiquitously distributed in mammalian tissues, are essential to control numerous physiological processes. The function of cyclic adenosine monophosphate (cAMP) is to activate protein kinase A (PKA), which in turn activates myosin light chain phosphatase, reducing phosphorylation and generating relaxation[18]. cAMP-activated PKA also activates and phosphorylates phosphodiesterase 4 (PDE4) isoforms which then degrade cAMP providing thus a pivotal acute feedback mechanism. Four genes (4A-D) encode a large number of PDE4 isoforms. The PDE4 family has gained interest in view of their involvement in the regulation of processes such as inflammation[19,20]. Nasal polyps or nasal polyposis is considered a subgroup of chronic rhinosinusitis, a common chronic inflammatory disease of the nasal mucosa. Experiments using knockout mice for PDE4D gene further supported PDE4D role in a regulation of airway smooth muscles contractility and recent genome-wide association study for asthma identified PDE4D as a highly plausible candidate gene[19]. In our sample size we have seen a statistically difference between the control group and patients with nasal polyps about the results of the PDE4D (rs1588265) gene.

RORA is located on chromosome 15q and spans approximately 730 kilobases[21]. It is known to play a key role in the regulation of circadian rhythms, bone morphogenesis, angiogenesis, and pathways including immunity/ inflammation, lipid metabolism, and cholesterol[22]. In addition data suggest an inhibitory role for RORA in inflammation and angiogenesis, cellular processes that have also been implicated in the development and progression of neovascular age-related macular degeneration[21,22]. Of these genes, RORA was selected for study due to its anti-angiogenic properties and regulation of immunity/inflammation. We did not see appreciable difference between the control group and patients with nasal polyps about RORA (rs11071559) gene. We did not see appreciable difference between peripheral blood in patients with nasal polyposis and control group about desmosomal junctions and RORA. We have seen a statically appreciable differences between the control group and patients with nasal polyps about the results of the PDE4D (rs1588265) gene. Further studies will provide a better understanding to evaluate association of this possibility.

Conclusions

Nasal polyps or nasal polyposis is considered a subgroup of chronic rhinosinusitis, a common chronic inflammatory disease of the nasal mucosa. The PDE4D family has gained interest in the complex pathogenesis of nasal polyposis. This is likely linked to the mucosal inflammatory response.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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