

Comparison of Expression of Cytokeratin 7 on Epithelium of Nasal Polyps and Mucosa of Chronic Rhinosinusitis

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ABSTRACT

Background and Objective: Nasal polyp is the most common benign mass of the nasal cavity and it is originated from nasal cavity or sinuses. Cytokeratins (CK) are intermediate filament proteins that provide mechanical support in epithelial cells. The purpose of this study was to compare the expression of cytokeratin 7 in epithelium of nasal polyps and nasal mucosa in chronic rhinosinusitis.

Materials and Methods: This research was a cross-sectional descriptive-analytic study. Level of expression of cytokeratin 7 in 90 samples from patients with nasal polyps and 30 samples from patient with chronic rhinosinusitis in Mostafa Khomaini hospital (Tehran, Iran) during 2007-2011 was assessed by immunohistochemistry using monoclonal antibody and Hematoxylin-Eosin staining and the results were analyzed by Mann-Whitney and t-tests.

Results: There was significant correlation between the level of expression of cytokeratin 7 in the epithelium of nasal polyps and nasal mucosa in chronic rhinosinusitis ($P < 0.05$). In other words, the level of expression of cytokeratin 7 in the epithelium of nasal polyp was higher than in nasal mucosa in chronic rhinosinusitis. There was also no correlation between the levels expression of CK7 in nasal polyp and other variable such as age and sex.

Conclusion: According to the significant correlation for the expression levels of CK7, it seems that this biologic factor is involved in the development of nasal polyp in the presence of inflammation.

1. Introduction

Nasal polyp is the most common chronic inflammatory disease of nasal cavity, affecting 1-4% of the total population (1). Its incidence increases with age and it is more common in adults (2). The diagnosis of nasal polyp is confirmed by endoscopy and computed tomography (CT) scan (3). The treatment of nasal polyp is based on topical and systemic corticosteroids and besides medical treatment, surgical treatment is also used (4).

Different mechanisms are involved in the development of nasal polyp such as allergy,

infection, mechanical obstruction, epithelial defects and autonomic disorders (2). The pathogenesis of nasal polyp is unclear, however, some studies in context have been done and there are some theories about this, for example, cytotoxic proteins from eosinophils, chemical, physical and immunological stimuli, changes in water and electrolyte transportation, cytokines and other factors (5). Another biologic factor that can be mentioned is cytokeratins and specific expression of cytokeratins have been used for the detection of epithelial cell (6).

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Chronic rhinosinusitis could affect quality of life and it can cause critical complications (7). Diagnosis of chronic rhinosinusitis is based on clinical signs and physical examination by speculum and endoscopy (8). Treatment of chronic rhinosinusitis is based on three principles: stop the growth of bacteria, reduction of swelling of the mucus, and drainage of the sinuses (7).

A better understanding of the pathogenesis of nasal polyps and its diagnosis is possible by using immunohistochemical methods that helps researchers to prevent and treat nasal polyp, as was designed in this study.

2. Materials and Methods

In this research, 120 patients participated and they are divided into 2 groups: the control group included 90 patients suffering from nasal polyp and the other group was case group including 30 patients with chronic rhinosinusitis. These patients underwent endoscopic surgeries in Mostafa Khomeini hospital (Tehran, Iran) during 2007-2011. The mean age of patients was 41.68 years. Patients' clinicopathologic data were collected from their files and analyzed. First of all, tissues were fixed in 10% buffered formalin for 24 hours, were embedded in paraffin, and then sectioned. We cut 3 micrometer thickness sections from paraffin block and put them on 2

slides. One of the slides was stained with Hematoxylin-Eosin and the other one was stained with immunohistochemical method using monoclonal antibodies. The percentage of positive cells for CK7 was counted by light microscopy. This study was double blind and 2 slides were stained with different code numbers and recognized by experienced pathologist.

2.1. Immunohistochemistry

First of all, deparaffinization was performed, then, endogenous peroxidase activity was blocked by using 0.3% hydrogen peroxide in citrate buffer (pH 6.0). To remove excess antibodies, the slides were covered with protein block solution. The next phase polymer solution (primary and secondary antibodies) was poured on slides. After these steps, the slides were covered with chromogen, then slides were placed in the Hematoxylin stain. The last step was dehydrating with alcohol, clarifying with xylene, and covering by mounting glue. Finally, specimens were examined and the percentage of CK7-positive cells in each sample was determined. According to Wauters et al (1995), immunohistochemical staining was evaluated (9) and it can be classified into five categories: negative (-) staining less than 5%, staining in 5-25% of cells (+), staining in 25-50% of cells (++), staining 50-75% of cells (+++), and staining more than 75% (++++)(Table 1).

Table 1: Evaluation of immunohistochemical staining intensity

Intensity of antibodies expression	Absent	Weak	Moderate	Strong	Severe
Grading	-	+	++	+++	++++

3. Results

Out of the 120 patients whom participated in this study, 74 (61/7%) were male and 46 (38/3%) were female and the average age of them was 41.68 years. In this study, we have 2 groups: the group with nasal polyp consisting of 90 patients and the other group consisted of 30 patients with CRS. The average age of patients with nasal polyp was 44.94 years and for patients with CRS, it was 31.9 years. Frequency distribution of

participants regarding degree of cytokeratin staining was as follows: there were not any people in grade 1 and most patients had grade 4 (54.17%). The percentage of staining of CK7 in the case and control groups was as follows: there were no patients in grade 1 in the control and case groups. Case (50%) and control groups (55.56%) had most patients in grade 4 (Table 2).

Table 2: The percentage of frequency distribution of cytokeratin 7 staining in case and control groups

	Control group	Case group	Total
Absent	0%	0%	0%
Weak	1.11%	6.67%	2.50%
Moderate	33.33%	43.33%	35.83%
Strong	55.56%	50%	54.17%
Severe	10%	0%	7.50%

According to the results and the use of analytic study by statistical tests, it was determined that there was significant correlation between the expression level of CK7 in the epithelium of nasal polyps and nasal mucosa in chronic rhinosinusitis ($P < 0.05$). We found out also that there was no statistically significant correlation between the intensity of expression level of CK7 and other variables such as age and sex.

4. Discussion

In this study, the expression level of cytokeratin 7 in 90 samples from patients with nasal polyp and 30 samples from patients with CRS were studied and the results showed that intensity of expression level of CK7 was higher in CRS.

The results of this study are along with results of Mitroei et al in 2011. In this study, the expression pattern of cytokeratins 7 and 20 in patients with chronic rhinosinusitis with nasal polyps were studied. Their research involved 106 patients and there was not any significant differences generated by origin environment or sex in the case of the patients with CRS with nasal polyposis. From histopathologic examination there are three type of nasal polyps: allergic, fibroinflammatory and hyplastic polyps. They are recognized that CK specific for overbasal cells decreases once the inflammatory process evolves, being less expressed in polyps with chronic inflammation. In this study, in addition, CK7 and CK20 were also investigated. Unlike our study, there was no

case group in this study, however, the same procedure was used (10).

Zhuo's research showed that CK7 and CK19 were the most important types of cytokeratin in the epithelium of the nasal polyps and CK19 is positive in all layers of covering epithelium and CK7 is positive in overbasal cells of pseudostratified epithelium. This research showed that during the process of inflammation, expression of the new cytokeratins begins while expression of other subtype of cytokeratin reduces. In this research, only nasal polyps were examined but in addition to CK7, other cytokertins such as CK 14, 19, and 20 were also examined (11).

From specific expression of cytokeratins, epithelial cells origin was evaluated by Hicks et al. In this study, expression of cytokeratins was evaluated in respiratory epithelial cell (REC) of nasal polyps and turbinates. It was clear that CK7, 8, 18, and 19 expressed in respiratory epithelial cell and respiratory epithelium had been found negative for CK20. Expression of CK7, 8, and 18 were found in basal cell of epithelium of nasal polyps while CK5 and 14 were expressed in basal cell of nasal epithelium. In this study, no case group was evaluated and it was concluded that the REC in nasal polyps and turbinates the same as the REC of lower respiratory airway (12). In this study, it was found out that the intensity of expression of CK7 in nasal polyp decreases compared with CRS. It appears that the process of inflammation may affect the differences of expression of CK7. According to significant correlation between the levels of expression of CK7, it seems that this biologic

factor may be involved in the development of nasal polyp in the presence of inflammation. To achieve desired results, more extensive studies are needed.

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