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ORIGINAL ARTICLE

Could Cellular Proliferation Be a Predictive Index for the Relapse of Nasal Polyposis and Down-Regulated by Nasal Steroid Treatment?

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Abstract The aim of this article is to identify the cellular mitotic activity using Ki-67 monoclonal antibody for predicting relapses of nasal polyposis after surgery. A prospective study was conducted at Kartal Training and Research Hospital Otolaryngology Department between January 2006 and September 2008. Nasal polyps were obtained from all patients and pathological materials were analyzed for the Ki-67 staining using immunohistochemistry. Patients were followed after surgery for 12 months for relapse. There was no statistically significant difference between recurrent and nonrecurrent polyps. Polyp recurrence has a multifactorial origin. Ki-67 index alone does not provide sufficient information about polyp recurrence before the operation.

Keywords Cellular proliferation · Nasal polyposis · Nasal steroid treatment

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Introduction

Nasal polyposis is a chronic inflammatory disease of the paranasal sinuses and nasal mucosa, severely affecting daily life. Although several studies have revealed the influence of cytokines, allergy, and environmental factors in polyp development, the etiology of nasal polyps is still unknown. According to most recent hypotheses, the key mechanism leading to inflammation and cellular proliferation is supposed to occur in the lamina propria. Secretory hyperplasia and squamous metaplasia are also observed in nasal polyps. These findings suggest a dysregulation of epithelial morphological changes [1].

Ki-67 is a monoclonal antibody which has been reported to detect cell-proliferation-associated human nuclear antigens present in the G1, S, S2 and M phases of the cell cycle [2]. Ki-67 has been applied to clinical material to assess the cell kinetics of excised tumors and has found a very important acceptance as a prognostic marker [3, 4].

The treatment of nasal polyposis should be tailored for each individual patient but most will require a combination of medical and surgical therapy to deal with relapses. Postsurgical medical treatment significantly reduces the number of recurrences, which is valuable in patients previously subjected to frequent polypectomies.

The aim of this study is to determine the value of Ki-67 monoclonal antibody as a predictive index for cellular proliferation in patients with relapsing nasal polyposis by use of immunohistochemistry and to investigate whether cellular proliferation can be down-regulated with nasal steroid treatment.

Patients and Methods

Nasal polyposis patients suffering from nasal obstruction and facial pain associated with mucopurulent rhinorrhea

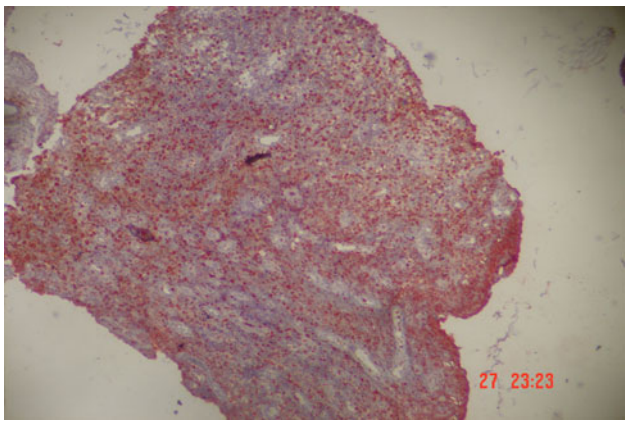

Fig. 1 Nasal polyp tissue dyed with Ki-67

were included in this study. Informed consent for study participation was obtained from all patients. Patients with a history of cystic fibrosis, primary ciliary dyskinesia or previous nasal surgery were excluded. Remaining patients were assigned to the study group ($n = 23$).

Patients' nasal symptoms and additional findings were recorded. Both sides of each patient's nose were examined with 4-mm 0° Hopkins rod endoscope and findings have been recorded. Nasal steroid treatment was given to patients pre- and post-operatively. Twenty-three polyp samples were collected during endoscopic functional sinus surgery for the treatment of polyposis. Inferior turbinate mucosa tissues were obtained as control samples from randomly selected patients ($n = 10$) suffering from nasal deviation and scheduled for septoplasty. The samples were stained with Ki-67 to detect cellular hyperproliferation of the mucosal and polyp tissues by use of immunohistochemistry. Twelve months later the patients in the study group were examined with 4-mm 0° Hopkins rod endoscope and according to the examination they were divided into two subgroups. Patients without recurrence of polyp were listed as the first group, and patients with recurrence of polyp were listed as the second group (Fig. 1).

Immunohistochemistry

All samples were formalin-fixed, and then paraffin embedded and 5 μm sections were obtained. Each section was systematically stained (hematoxylin-eosin) for standard histomorphological analysis.

Before immunostaining, deparaffinized tissue sections were boiled in citrate-buffer for enhanced antigen retrieval in formalin fixed, paraffin-embedded tissues, we performed the microwave-oven heating method. The deparaffinized sections were soaked in citrate buffer and heated in a microwave oven at 750, 350, 160 W, s minutes, 30 s, and 5 s respectively. Horse raddish peroxidase (HRP) of each section was inhibited with 3% hydrogen peroxide in methanol for 20 min. Each section was incubated for 90 min with primary antibody which was a Rabbit Anti-Ki67 Monoclonal Antibody (Clone SP6) (Neomarkers, USA).

After being washed with PBS (phosphate buffer saline), the procedure was finished with routine staining.

Quantitative and Statistical Analysis

Only epithelial cells with a clear nuclear immunostaining were counted as positive. The number of positive cells with a nuclear pattern within each microscopic field at a magnification of 400 was counted. Three high-power fields at a magnification of 400 were counted. The number of nuclei observed ranged from 150 to 300 in every case. The Ki-67 labeling index was determined by the quotient of Ki-67 positive cells and total number of cells evaluated. Statistical significant differences were established using Kruskal–Wallis and Mann–Whitney U tests. $p < 0{,}05$ was considered significant.

Results

Study group included a total of 23 patients affected by nasal polyposis with no history of cystic fibrosis or primary ciliary dyskinesia (14 females and 9 males with a mean age of 34 years, range: 15–53 years). The control group included 10 patients presenting with nasal septal deviation (5 females and 5 males with a mean age of 36 years, range: 18–49 years).

The proportion of Ki-67 positive nuclei in the nasal polyp epithelium showed large individual variation, ranging from 2, 12–73, 52%. Inferior turbinate mucosa samples showed 10.5–15% Ki-67 positive cells. Polyp recurrence was seen in 7 patients 12 months later. 16 patients had no recurrence. Ki-67 expression could not be detected in one patient with polyp recurrence and three patients with no recurrence. The staining ratio of the two groups of the nasal polyposis was given at Table 1. The average percentages of Ki-67 positive cells in inferior turbinate mucosa and nasal polyp epithelium with or without recurrence have been summarized in Table 2. Nasal polyp sample groups showed no significant difference ($p = 0{,}678$) (Fig. 2).

Table 1 Staining ratio of the two groups of nasal polyposis patients

	Staining (−)		Staining (+)		p
Polyp without recurrence (group 1)	3	18.8	13	81.3	1.000
Polyp with recurrence (group 2)	1	18.8	6	85.7	

Table 2 Percentages of Ki-67 positive cells in control mucosa and polyp epithelium

	Polyp Ki-67		*p*
	Median	Mean ± S.D.	
Polyp without recurrence (group 1)	17.52	20.07 ± 15.52	0.678
Polyp with recurrence (group 2)	29.25	29.79 ± 26.84	
Control group (inferior turbinate mucosa)	13.95	13.35 ± 2.02	

Discussion

Recurrent nasal polyposis is one of the most common unsolved problem in clinical rhinology. In the last few years a great number of histopathological, immunohistochemical and immunological studies on nasal polyps have been carried out by several authors. Many data suggest that the presence of polyps is the result of various inflammatory, allergic and pseudo-allergic processes which finally lead to the formation of the edema constitutive of the polyp itself [5].

The elevated numbers of activated eosinophils, neutrophils and plasma cells in nasal polyps compared with nasal mucosa has been reported. These data suggest that inflammatory processes may play important roles in the pathophysiology of nasal polyposis [6].

In nasal polyposis, inflammatory reactions could stimulate epithelial proliferation. Inflammatory cells are able to produce some growth factors like epidermal growth factor, transforming growth factor, insulin-like growth factor-I. These growth factors could stimulate epithelial proliferation [7]. A very similar mechanism has been proposed in cholesteatoma [8]. In cholesteatoma several growth factors have been identified. Due to inflammatory reaction, these factors were released from the inflammatory cells and this could induce the cell proliferation.

Ki-67 monoclonal antibody was used to detect proliferation of epithelial cells. Significantly increased epithelial cell proliferation was noted in nasal polyps compared to sinonasal mucosa [9]. The same findings were reported by different authors [1, 10]. Although suitable surgical and medical treatments were performed, the recurrence of the polyps could not be prevented. A few study reported about the detection of the recurrent polyps proliferative activity [11]. By using flow cytometry, the percentage of cells in the S phase is found at higher concentration in patients suffering from relapsing polyposis. It is reported that hyperproliferative ability is higher at recurrent polyps [11]. Nasal polyps with recurrent disease displayed higher scores for proliferation markers. In recurrent disease some increase in proliferation activity and some changes in the parameters of the DNA analysis occurred, indicating more aggressive behavior [12]. Ki-67 was detected and compared between two groups as a proliferation index.

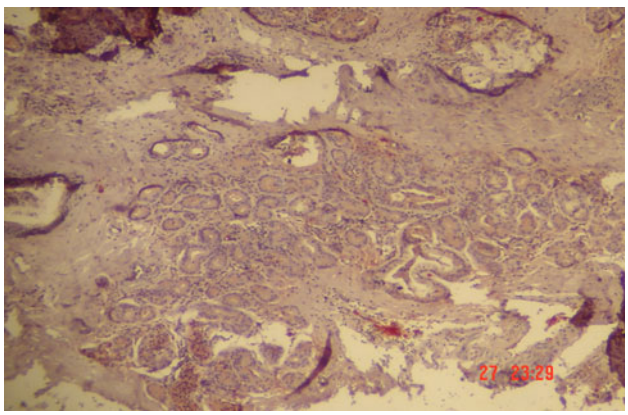

Fig. 2 Inferior turbinate mucosa dyed with Ki-67

Kosem et al. [13] reported that proliferative activity in the surface epithelial cells of recurring nasal polyps is significantly higher than that in nonrecurring nasal polyps. Contrary to their results, statistical analysis of our data shows no relation between polyp recurrence and Ki-67 activity. However, although statistically insignificant, the patients with a higher Ki-67 staining ratio developed recurrence of nasal polyposis.

Nasal polyposis is a multifactorial entity. Several prognostic factors may play a role in the development of the disease and the recurrences such as the presence of bilateral involvement of the sinus system, involvement of more than one subside (anterior ethmoid, posterior ethmoid, maxillary sinus, sphenoid), ASA and NSAID intolerance and abundant eosinophilic infiltration in the mucous chorion [14]. This suggests that recurrence of the polyps does not depend solely on cellular hyperproliferation.

In our study, preoperative steroid treatment might have resulted in inhibition of the inflammatory response and growth factor release and thus the proliferative activity of polyps. Therefore Ki-67 index alone does not provide sufficient information about polyp recurrence before the operation. Other etiologic factors listed above must also be considered in patients with recurrent nasal polyposis. New immunohistochemical studies taking preoperative and postoperative medical treatment, involvement of sinuses, presence of ASA and NSAID intolerance or abundant eosinophilic infiltration into consideration are needed in the future.

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