

Immune activity of Ki-67 during nasal polyp development

B. HAZNEDAR¹, Ö. KAPLAN², F. AŞIR², I.S. ERMIŞ³

¹Division of Otolaryngology, Gazi Yaşargil Training and Research Hospital, Diyarbakır, Turkey

²Department of Histology and Embryology, Medical School, Dicle University, Diyarbakır, Turkey

³Department of Gynecology and Obstetrics, Medical Faculty, Harran University, Şanlıurfa, Turkey

Abstract. – OBJECTIVE: Nasal polyps are non-cancerous, soft painless growth of nasal mucosa. In this study, our aim was to investigate the Ki-67 expression level in nasal polyps by immunohistochemical method.

PATIENTS AND METHODS: 30 patients with nasal polyps were included in this study. Nasal polyps were processed for paraffin wax embedding protocol. Samples were fixed and embedded in paraffin blocks. 5 µm sections were stained with Hematoxylin-Eosin dye and immune stained with Ki-67 antibody. Sections were analyzed under light microscope.

RESULTS: Blood parameters showed that white blood cells, hematocrit and platelet were higher than normal range. In sections of hematoxylin-eosin staining, elevated basal cells, thin basement membrane, leukocyte infiltration, collagen fibers degeneration were observed. Masson trichrome staining revealed that degenerative epithelial cells, detached basement membrane and edema were observed. Ki-67 expression was observed in mucosal epithelial cells, vascular endothelial cells and plasma cells in immune staining.

CONCLUSIONS: Epithelial degeneration in nasal polyps and leukocyte infiltration induce nasal adenoma. Ki-67 expression may be a diagnostic tool for epithelial leukocyte formation.

Key Words:

Nasal polyp, Ki-67, Inflammation, Degeneration.

Introduction

Nasal polyps are benign tumors that develop as a result of inflammation in the nasal mucosa. The prevalence of nasal polyps is between 2-4% in European countries¹. Polyps are round or oval shaped structures that usually originate from the ethmoid sinuses. It becomes evident as a result of edema and hyperemic areas in the nose during the examination². The easiest way to diagnose nasal polyps is by radiographic examination.

However, paranasal tomography is recommended for the stage of the polyp, the condition and evaluation of the sinuses³.

Although the pathogenesis of nasal polyps has not been fully determined, genetic predisposition, allergies, chronic sinusitis and rhinitis, nasal mucosal vascular disorders, inflammation and mucosal irritation are among the risk factors⁴. Although the course of the disease is generally seen after the age of 20, young children may develop the disease with genetic diseases, such as Kartagener's syndrome or Cystic Fibrosis⁵. Treatments such as drug administration or surgical intervention are applied in the treatment of nasal polyps. The main goals of treatment are removal of the polyp, regulation of nasal airflow, improvement of the sense of smell and rhinitis symptoms⁶.

Ki-67 is a cellular marker used in the prognosis of many tumors and its expression is increased in proliferating cells. It also has many molecular functions in proliferating cells and its roles in mitosis. It is widely used for the diagnosis of cancer by immunohistochemistry⁷. Fan et al⁸ used it as an immune marker to evaluate the proliferative activities of nasal polyps and papilloma. In their study, they reported that the expression of Ki-67 increased compared to normal mucosa. Kösem et al⁹ examined the expression of Ki-67 in patients with recurrent and non-recurrent nasal polyps. They found the mean Ki-67 expression to be higher in recurrent patients than in patients with non-recurrent nasal polyps.

In this study, the importance of inflammation pathway Ki-67 in nasal polyp that develops as a result of different reasons and affects respiration is explained.

Patients and Method

Ethical approval was obtained from Diyarbakır Gazi Yaşargil Training and Education Hos-

pital, Clinical Trials Ethical Committee (Date: 20/01/2023, record number: 48). In our study, samples were taken from 30 patients with nasal polyps from Gazi Yaşargil Training and Research Hospital Otorhinolaryngology Clinics. Informed consent forms were signed by all patients.

Histopathologic Analysis

Nasal polyps were fixed with zinc-Formalin solution (catalog no: Z2902, Sigma-Aldrich, St. Louis, MO, USA) and washed under tap water by 10 minutes. Tissues were passed through ascending alcohol series for about 24 hours. Tissues were washed with xylene 2x15 minutes and incubated within paraffin wax. 5 µm sections were cut with microtome (catalog no: Leica RM2265, Wetzlar, Germany). Deparaffinized within xylene for 2X15 minutes, sections were brought to distilled water. Some of the sections were stained with routine Hematoxylin and Eosin, the rest were kept for immunohistochemical staining¹⁰.

Immunohistochemical Analysis

Nasal polyps were brought to distilled water. Hydrogen peroxide solution (catalog No.: TA-015-HP, ThermoFischer, Fremont, CA, USA) were dropped on sections for 15 minutes. After washing in PBS for 3X5 minutes, ultra-V Block (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, USA) was applied to sections for 6 minutes. Sections were incubated with primary antibody anti-Ki-67 (catalog no: ab16667, Abcam, Cambridge, MA, USA) at +4 °C overnight. Sections were allowed to warm at room temperature for 30-60 minutes. Sections were washed with biotinylated secondary antibody (catalog No.: TP-015-BN, ThermoFischer, Fremont, CA, USA) for 10 minutes. Streptavidin-peroxidase (catalog No.: TS-015-HR, ThermoFischer, Fremont, CA, USA) was dropped onto sections for 10 minutes. Clearing with PBS, DAB (catalog No.: TA-001-HCX, ThermoFischer, Fremont, CA, USA) was used as chromogen. Sections were counter stained with Gill hematoxylin (catalog No.: 105174, Sigma-Aldrich, St. Louis, MO, USA), and mounted with entellan (catalog No.: 107961, Sigma-Aldrich, St. Louis, MO, USA). Slides were analyzed with Zeiss Imager A2 Zen 3.0 software (Germany, Carl-Zeiss-Straße, Oberkochen, Germany) and photomicrographed¹¹⁻¹⁴.

Statistical Analysis

Statistical analysis was done with SPSS version 24 (IBM Corp., Armonk, NY, USA). In-

dependent variables were shown as mean ± standard deviation. Significance level (*p*) was accepted <0.05.

Results

Biochemical Findings

Nasal polyps cause inflammation in nasal mucosa and induce inflammatory pathway. Due to inflammatory response, an increase in white blood cell, hematocrit and platelet levels were observed. Blood parameters of patients and their graphical illustration were shown in Table I and Figure 1-3.

Histopathological Findings

In the histopathological examination of nasal polyp sections, an increase in rod-like cells from the germinative layer of the oral epithelium to the basal region, thinning of the basement membrane and some atypical cell-like structures were observed. In the lamina propria region, there were aggregated and solitary dispersed leukocytes and small dispersed plasma cells. In some areas, collagen degeneration was evident and separations in the basement membrane were detected (Figure 4a). In a special staining (Trichrome-Masson staining), some degenerative cells were detected in the epithelium, while atypical cells with a stellar appearance were detected in places.

A typical looking rod-like cells were particularly striking. Although some detachments were observed in the basement membrane, an increase was observed in the congested structures with intact integrity. In the connective tissue struc-

Table I. Blood parameters of patients.

Parameters	mean ± SD
WBC (10 ⁹ /L)	7.84 ± 2.19
NEU (10 ³ /uL)	4.53 ± 1.89
PLT (10 ⁹ /L)	283.80 ± 63.09
HCT (%)	44.12 ± 5.58
HGB (g/dL)	14.44 ± 1.85
CRP (mg/dL)	1.78 ± 2.87
PT-INR	1.05 ± 0.11
PT (sec)	11.78 ± 1.12
Glucose (mg/dL)	94.41 ± 25.95
ALT (U/L)	17.19 ± 8.5
AST (U/L)	17.70 ± 5.29

WBC: white blood cell, NEU: neutrophile, PLT: platelet, HCT: hematocrit, HGB: hemoglobin, CRP: C-reactive protein, PT-INR: prothrombin time - international normalized ratio, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Figure 1. Mean of white blood cells (WBC), neutrophile (NEU), C-reactive protein (CRP) and prothrombin (PT-INR).

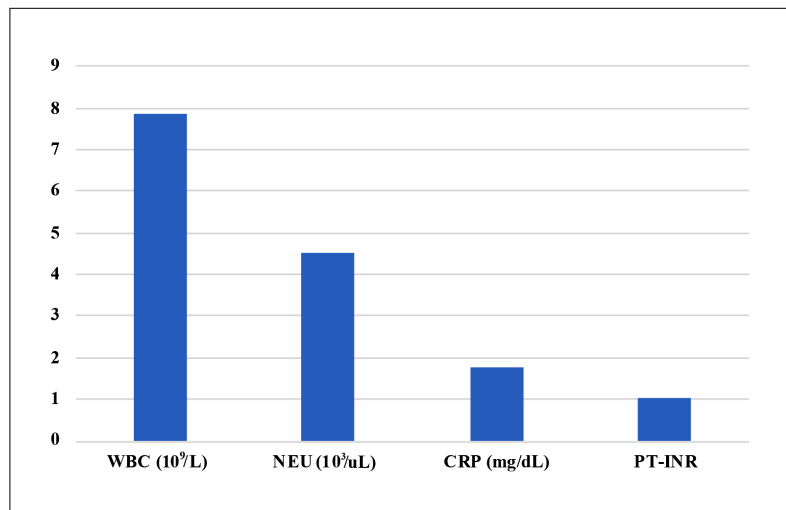


Figure 2. Mean of hematocrit (HCT), hemoglobin (HGB), prothrombin time (PT), ALT (alanine aminotransferase), AST (aspartate aminotransferase).

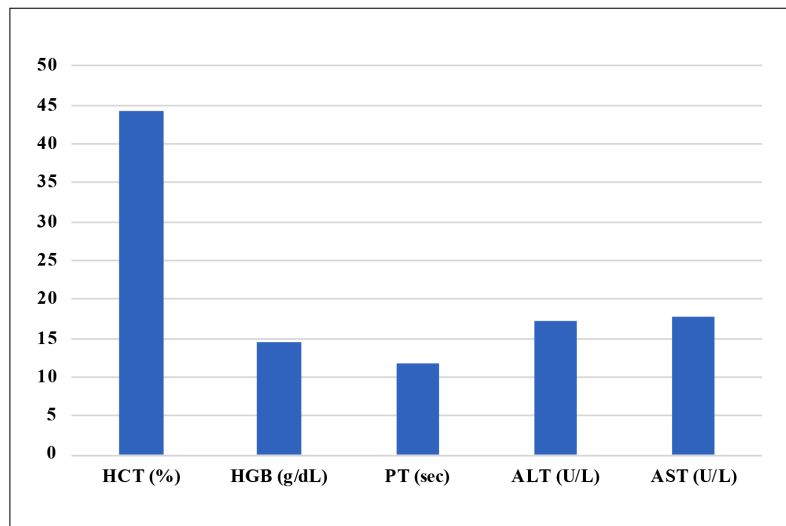
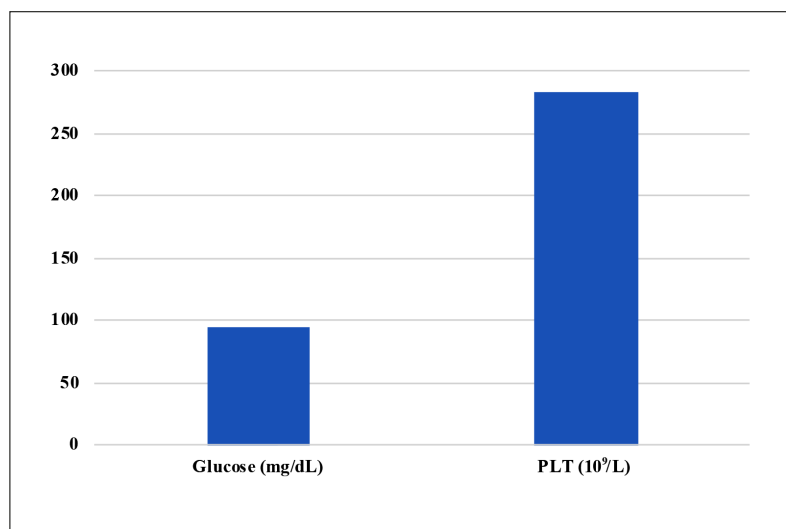


Figure 3. Mean of glucose and platelets (PLT).



tures, especially in the collagen fibers, edema and intense inflammatory cell infiltration in the form of aggregates were observed together with local degenerative changes. In general, changes due to intense inflammation were observed in the epithelial and connective tissue areas (Figure 4b). In Ki-67 immunohistochemical activity, Ki-67 expression with some degenerative cells, especially in the germinative region of Oral Mucous epithelium, was positive. Positive Ki-67 was observed in cell infiltrations in the form of small aggregates in a solitary fashion just below the basal lamina. Ki-67 expression was positive in endothelial cells located above dilated vessels and thinned basement membranes, and positive expression was observed in plasma cells in some areas (Figure 4c).

Discussion

Nasal polyps, soft, pale in color, hanging into the nose, painless to touch, are also called a type of chronic sinusitis. It has been reported that a large number of active eosinophils, neutrophils and plasma cells appear in the nasal mucosa in nasal polyps, which can stimulate epithelial proliferation with their inflammatory reactions. Inflammatory cells can stimulate epidermal growth factor, transforming growth factor, insulin-like growth factor-I epithelial proliferation¹⁵. Although nasal polyps are divided into three as allergic, edematous and eosinophilic, they are generally

seen as binary combinations. Macroscopically, there is softening of the mucosa, a white color, fibrosis and an ulcerated appearance¹⁶. Histopathologically, there may be several glands and intense edema as a group, or the glands may be scattered. There are irregularities and enlargements in the structure of the glands, absence of seromucous glands in the turbinates, inflammatory cell infiltration with common eosinophils, and lymphocytes in the stroma. Hyperplasia in goblet cells, thickening of the basement membrane and hyalinization are other pathological findings^{15,17}. In our study, aggerate and solitary inflammatory cell infiltration was observed in the epithelial periphery of the gland groups, congestion in the blood vessels in the lamina propria, dysplasia in the endothelial cells, and degeneration in the gland cells (Figure 4a and 4b).

Ki-67 is a nuclear protein found in proliferating cells. It is usually seen in the G1, S, M and G2 phases. It is not in the G0 phase. It is a protein that shows the morphological features of cell proliferation well, and is a protein used in mitotic index and tumor staging^{18,19}. Viksne et al²⁰ studied Ki-67 expression in patients with nasal polyps and inverted papilloma. The authors claimed that the Ki-67 index in nasal epithelial cells was higher in the inverted papilloma group compared to patients with nasal polyps, and that Ki-67 played a role in the pathogenesis of these two diseases. Viksne et al²⁰ investigated Ki-67 proliferation marker in their pilot study in patients with rhinosinusitis and nasal polyps. They stated that

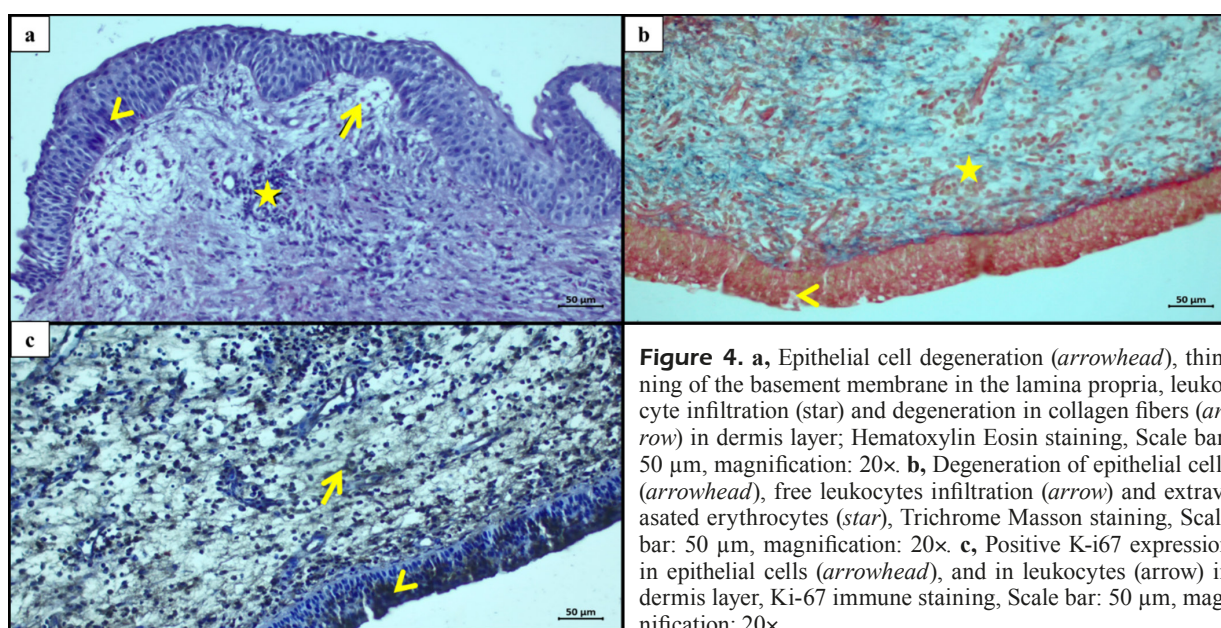


Figure 4. **a**, Epithelial cell degeneration (*arrowhead*), thinning of the basement membrane in the lamina propria, leukocyte infiltration (*star*) and degeneration in collagen fibers (*arrow*) in dermis layer; Hematoxylin Eosin staining, Scale bar: 50 µm, magnification: 20×. **b**, Degeneration of epithelial cells (*arrowhead*), free leukocytes infiltration (*arrow*) and extravasated erythrocytes (*star*), Trichrome Masson staining, Scale bar: 50 µm, magnification: 20×. **c**, Positive Ki-67 expression in epithelial cells (*arrowhead*), and in leukocytes (*arrow*) in dermis layer, Ki-67 immune staining, Scale bar: 50 µm, magnification: 20×.

compared to the control group, Ki-67 expression increased in epithelial cells of patients with nasal polyps and decreased in connective tissue.

Epithelial damage in nasal polyps and prolapse of the lamina propria as a result of localized inflammatory cells and edema can be explained by the re-epithelialization of the tissue and the development of mature polyp formation²¹. In our study, the presence of atypical looking cells among epithelial cells, leukocytes, and small aggregates of cells induced inflammation. It has been thought that the inflammatory response, which can directly affect the airways or affect the activity of other inflammatory cells, may play an important role in regulating the local immune response by releasing epithelial inflammatory mediators and expressing various cell adhesion molecules (Figure 4a and 4b).

The dynamic localization of Ki-67 has led to suggestions that it may coordinate nucleolar fragmentation and recombination on both sides of mitosis²². Indeed, Ki-67 is required to localize granular nucleolar components to mitotic chromosomes, potentially playing a role in nucleolar dissociation between daughter cells²³. Inflammation is a physiological reaction to cell and tissue damage caused by trauma, ischemia, infection, and other pathological conditions. Elevated white blood cell count (WBC) and varying levels of other acute phase reactants are the main signs of inflammation²⁴⁻²⁷. It showed a consistent pattern of baseline responses involving co-regulation of the WBC and platelets (PLT) populations: uncomplicated improvements are with exponential decay from the maximum WBC followed by delayed linear growth of the PLT. In our study, the increase in white blood cell, hematocrit level and platelet levels, which developed due to the increase in inflammation (Table I, Figure 1-3), showed parallelism with the increase in Ki-67 level (Figure 4c). It has altered human acute inflammatory healing and trajectory, defined by disruption of WBC, and delayed linear increase in PLT. Leukocytosis is an important sign of inflammation²⁸⁻³¹.

Conclusions

It has been observed that epithelial degeneration, which begins with the formation of nasal polyps, and the appearance of leukocyte structures in the epithelium can accelerate the development of nasal adenoma and increase leukocyte migration and T lymphocyte aggregation with inflammation.

In addition, it was observed that it induced the expression of KI-67, which is an immune stimulant. It has been thought that the KI 67 signal in the nasal polyp may be a marker of epithelial leukocyte formation.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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The study didn't receive any funding.

Authors' Contribution

Conceptualization: BH, FA; Methodology: BH, ÖK; Validation: FA; Formal analysis: BH, FA; Investigation: BH, FA, ÖK; Resources: FA, ÖK; Data Curation: BH, ÖK; Writing - Original Draft: BH, FA, ÖK; Writing - Review & Editing: FA; Visualization: FA, ÖK; Supervision: BH, FA; Project administration: BH, FA.

Informed Consent

Informed consent forms were signed by all the patients included in this study.

Ethics Approval

Ethical approval was obtained from Diyarbakır Gazi Yaşargil Training and Education Hospital, Clinical Trials Ethical Committee (Date: 20/01/2023, record number: 48).

ORCID ID

B. Haznedar: 0000-0002-4990-5260; F. Aşır: 0000-0002-6384-9146; Ö. Kaplan: 0000-0001-5203-9650; I.S. Ermiş: 0000-0002-9714-4670.

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