ORIGINAL ARTICLES

UDC: 616.211-006.5-091.8
https://doi.org/10.2298/VSP171228139T

Nasal polyposis: a semiquantitative morphometric histopathological study

Nazalna polipoza: semikvantitativna morfometrijska patohistološka studija

Aleksandar Trivić*†, Nada Tomanović†‡, Sanja Krejović Trivic*†, Jovica Milovanović*†, Ivan Boričić†‡, Ana Jotić*†, Miljan Folić*†, Ivana Ćolović Čalovski†§, Nikola Miković‖, Zoran Tatić¶

Clinical Centre of Serbia, *Clinic for Otorhinolaryngology and Maxillofacial Surgery, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, ‡Institute of Pathology, §Institute of Microbiology and Immunology, Belgrade, Serbia; University of Belgrade, Faculty of Dentistry, ‖Clinic for Maxillofacial Surgery, Belgrade, Serbia; Military Medical Academy, ¶Dental Clinic, Belgrade, Serbia; University of Defence, **Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Nasal polyps are inflammatory hypertrophic proliferations of the sinonasal mucosa composed of both epithelial and stromal elements. The aim of this study was to determine histopathological hallmarks of nasal polyposis via semiquantitative morphometric study. **Methods.** The study comprised 77 patients with chronic rhinosinusitis and nasal polyposis (CRSwNP) that underwent functional endoscopic sinonasal surgery performed by the same surgeon. The control group consisted of 9 different nasal mucosal samples that were taken from patients without CRSwNP that underwent functional and esthetic surgery. Morphometric analysis included gradation of tissue edema within polyps, thickening of epithelial basal membrane, degree of inflammation, presence/absence of metaplasia within epithelium, degree of fibrosis within polyps, and percentage of inflammatory cells within inflammatory infiltrate (lymphocytes, macrophages, plasma cells, neutrophils and eosinophils). **Results.** As expected, samples from the study group showed significantly higher degree of inflammation than samples from the control group ($\chi^2 = 35.89$, with $p < 0.01$). Degree of fibrosis in nasal polyposis was in positive correlation with duration of symptoms ($r = 0.25$, $p < 0.05$) and with percentage of macrophages in inflammatory infiltrate ($r = 0.26$, $p < 0.05$). Patients with nasal polyposis had significantly lower number of lymphocytes ($r = -7.66$, $p < 0.01$), but significantly higher number of eosinophils ($r = 3.84$, $p < 0.01$), macrophages ($r = 3.34$, $p < 0.01$) and plasma cells ($r = 3.14$, $p < 0.01$) than controls ($p < 0.01$). **Conclusion.** Tissue samples from patients with nasal polyposis show significant changes that reflect in various degrees of inflammation, fibrosis and basement membrane thickening which may contribute to more difficult surgical management and perioperative complications such as bleeding.

Key words:
nasal polyps; otorhinolaryngologic surgical procedures; postoperative complications; histology.

Apstrakt

Uvod/Cilj. Nazalni polipi predstavljaju inflamatorne izrasline hipertrofične respiratorne sluznice i sačinjeni su od epitelnih i stromalnih elemenata. Cilj ove studije bio je da odredimo patohistološka obeležja nazalnih polipa kroz semikvantitativnu morfometrijsku studiju. **Metode.** Izvršena je semikvantitvna morfometrijska analiza uzoraka nazalne sliznice uzetih od 77 bolesnika sa hroničnim rinosinuzitisom i nazalnim polipima. Kontrolnu grupu sačinjavali su uzorci nazalne sluznice, uzeti od 9 pacijenta bez nazalne polipoze koji su bili podvrgnuti funkcionalnoj i estetskoj hirurgiji. Kod svih bolesnika je učinjena funkcionalna endoskopska sinonazalna hirurgija od strane istog hirurga**.** Morfometrijska analiza je uključivala gradaciju edema tkiva sa polipima, debljinu bazalne membrane, stepen inflamacije, prisustvo/odsustvo metaplazije u epitelu, stepen fibroze, kao i procenat zapaljenskih ćelija sa zapaljenskim infiltratom (limfocite, makrofage, plazma ćelije, neutrofile i eozinofile). **Rezultati.** Kao što je i očekivano, uzorci iz ispitivane grupe su imali značajno veći stepen inflamacije u odnosu na kontrolnu grupu (χ^2

Correspondence to: Aleksandar Trivić, Clinical Centre of Serbia, Clinic for Otorhinolaryngology and Maxillofacial Surgery, Pasterova 2, 11 000 Belgrade, Serbia. E-mail: drcole@sbb.rs

= 35.89, $p < 0.01$). Stepen fibroze kod polipa nosa je bio u pozitivnoj korelaciji sa trajanjem dužine simtoma ($r = 0.25$, $p < 0.05$) i sa procentom makrofaga u zapaljenskom infiltrate ($r = 0.26$, $p < 0.05$). Bolesnici sa nazalnom polipozom imali su značajno veći broj limfocita ($r = -7.66$, $p < 0.01$), ali i značajno veći broj eozinofila ($r = 3.84$, $p < 0.01$), makrofaga ($r = 3.34$, $p < 0.01$) i plazma ćelija ($r = 3.14$, $p < 0.01$) nego kontrolna grupa ($p < 0.01$). **Zaključak.** Uzorci tkiva kod bolesnika sa nazalnom polipozom pokazuju značajne promene koje se ogledaju u različitom stepenu inflamacije, fibroze i zadebljanja bazalne membrane što može značajno otežavati hirurški zahvat, kao i uticati na veći stepen perioperativnih komplikcija kao što je krvarenje.

Ključne reči:
nos, polipi; hirurgija, otorinolaringološka, procedure; postoperativne komplikacije; histologija.

Introduction

Nasal inflammatory polyps are nonneoplastic proliferations of the sinonasal mucosa composed of both epithelial and stromal elements. The pathogenesis of these lesions is still uncertain; however, mucosal edema and inflammation, cytokine secretion, and collagen synthesis stimulated by eosinophils have all been implicated [1–3]; polyps are frequently associated with salicylates intolerance, asthma and cystic fibrosis [1–9]. Symptoms at presentation include nasal obstruction, rhinorrhea, headache, impaired sense of smell and postnasal discharge [1–6]. Nasal polyposis (NP) is slightly more prevalent in men, with an incidence in the fifth decade of life, and affects between 1% and 4% of the population [5].

Patients who have failed medical management may benefit from surgical intervention in the form of transnasal ethmoidectomy or, more recently, functional endoscopic nasal surgery. Even after appropriate surgical therapy, a significant number of patients with chronic rhinosinusitis (CRS) with NP (CRSwNP) experience recurrences [9], with disease-free interval significantly shorter in patients with eosinophilic-type polyposis. NP often present as multiple bilateral masses arising from the lateral nasal wall. Inflammatory polyps can measure up to several centimeters in diameter, with usually a broad stalk and have a myxoid or gelatinous appearance with a smooth surface. Histologically, they are lined with respiratory epithelium with a variably thickened basement membrane. The epithelium often exhibits some degree of squamous metaplasia. The stroma is abundant and highly edematous or myxoid and contains a mixed inflammatory infiltrate composed of eosinophils, lymphocytes, and plasma cells. Sometimes Charcot-Leyden crystals associated with abundant eosinophils may be seen. These crystals are a result of eosinophil degeneration and are formed at the surface of nasal mucosa and within mucus. In cases associated with infection, neutrophils may be present in large numbers. The stroma contains a variable number of fibroblasts and blood vessels [1].

The aim of this study was to determine histopathological hallmarks of nasal polyposis via semiquantitative morphometric study.

Methods

We conducted a study during period of January 1st, 2016 until December 31st, 2016. The study comprised 77 patients with CRSwNP that underwent endoscopic sinus surgery performed by the same surgeon. Patients had no history of cystic fibrosis, antrochoanal polyp or primary ciliary dyskinesia. Nasal steroid treatment was given to patients pre and postoperatively. Nasal polyps were sent for histopathological examination. Representative tissue samples were processed routinely, were formalin-fixed and paraffin embedded. Tissue sections that were 5 μm thick were made and stained with hematoxillin & eosin. The control group consisted of 9 different nasal mucosal samples that were taken from patients without CRSwNP that underwent functional and esthetic surgery. The samples of mucosa were taken from inferior nasal concha. After the histopathological diagnosis of nasal polyposis was established, semiquantitative morphometric analysis was performed. It included gradation of tissue edema within polyps according to the degree of lamina propria expansion (0 - no edema, 1-slight edema/slight lamina propria expansion, 2 - moderate edema/moderate lamina propria expansion, 3 - severe edema/marked lamina propria expansion), thickening of epithelial basal membrane (0 - no thickening, 1 - slight thickening, 2 - moderate thickening, 3 - severe thickening), degree of inflammation (0 - no inflammation, 1 - slight inflammation with inflammatory infiltrate comprising less than 30% of the sample/per 100 x magnification, 2 - moderate inflammation, with inflammatory infiltrate comprising between 30% and 60% of the sample/per 100 x magnification, 3 - severe inflammation, with inflammatory infiltrate comprising more than 60% of the sample/per 100 x magnification), presence/absence and type of metaplasia within epithelium (goblet cell metaplasia and squamous metaplasia), degree of fibrosis within stroma (0 - no fibrosis, 1 - slight fibrosis that comprises less than 30% of stromal surface, 2 - moderate fibrosis that comprises up to 50% of stromal surface, 3 - severe fibrosis that comprises more than 50% of the stromal surface), and percentage of inflammatory cells within inflammatory infiltrate (lymphocytes, macrophages, plasma cells, neutrophils and eosinophils). We also evaluated gender and age in both the control and the study group, duration of symptoms, prior history of allergies and polyposis laterality. We did not evaluate the percentage of eosinophils within nasal mucus. Analysis was performed using a Cell F imaging analysis programme and was performed by one pathologist.

Data were analyzed by the χ^2-test, Pearson's correlation coefficient and *t*-test with p values ≤ 0.05 that were considered significant. All analyses were done in the software package Statistical Package for Social Sciences 18 (SPSS 18).

Results

Our study included 77 patients, 46 (59.7%) male and 31 (40.3%) female. Control group consisted of 9 patients, 6 (66.7%) male and 3 (33.3%) female. Average age (Table 1) in the study group was 45.40 ± 14.92 years (age ranged from 13 to 71 years). Duration of symptoms ranged from 1 to 31 months, the average being 12.10 ± 6.81 months. Majority of patients (89.6%) had bilateral NP. We found no gender differences in our patients in comparison with any of examined morphological data. Samples from the study group showed significantly higher degree of inflammation than samples from the control group ($\chi^2 = 35.89$, $p < 0.01$). Slight inflammation was found in 35 patients, moderate in 33 patients and severe in 9 patients from the study group (Figure 1). Fibrosis (Figure 2) was slight in 13 patients, moderate in 34 and severe in 17 patients within the study group, whilst 17 showed no morphological signs of fibrosis. Degree of fibrosis in NP was in positive correlation with duration of symptoms ($r = 0.25$, $p < 0.05$) and with percentage of macrophages in inflammatory infiltrate ($r = 0.26$, $p < 0.05$). There was no such correlation between degree of tissue edema and age/duration of symptoms. There were no patients with 50% or more macrophages in the inflammatory infiltrate. Patients with NP had significantly lower number of lymphocytes ($r = -7.66$, $p < 0.01$), but significantly higher number of eosinophils ($r = 3.84$, $p < 0.01$), macrophages ($r = 3.34$, $p < 0.01$) and plasma cells ($r = 3.14$, $p < 0.01$) than the controls ($p < 0.01$).

Table 1
Clinical characteristics of the study group with nasal polyposis and the control group

Characteristics	Study group	Control group
Gender, n (%)		
male	46 (59.7)	6 (66.7)
female	31 (40.3)	3 (33.3)
Total	77 (100)	9 (100)
Age (years), min–max.	13–71	18–55
Duration of symptoms (months), min–max . (mean ± SD)	1–31 (12 ± 6.81)	-

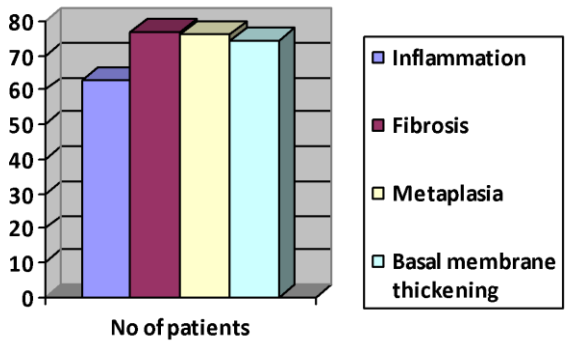

Fig. 1 – Histopathological hallmarks of the study group with nasal polyposis.

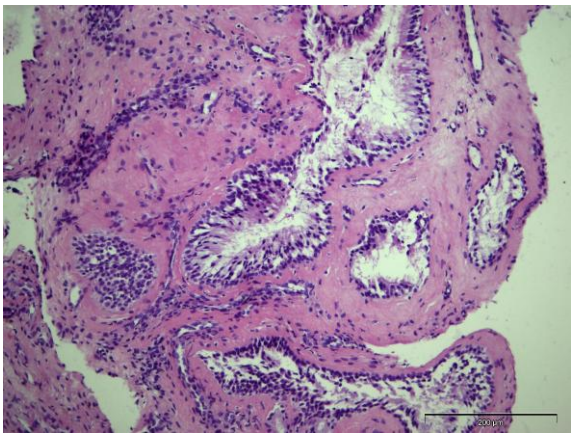

Fig. 2 – Nasal polyp with mild stromal fibrosis (hematoxillin & eosin, original magnification ×200).

Epithelial metaplasia was found in a great majority of patients: isolated goblet cell metaplasia in 70.1% (Figure 3) and combined goblet cell and focal squamous metaplasia in 26%. Only 1 (1.3%) patient showed no adaptive epithelial changes. We also found no correlation of basal membrane thickening (Figure 4) with age of patients and duration of symptoms.

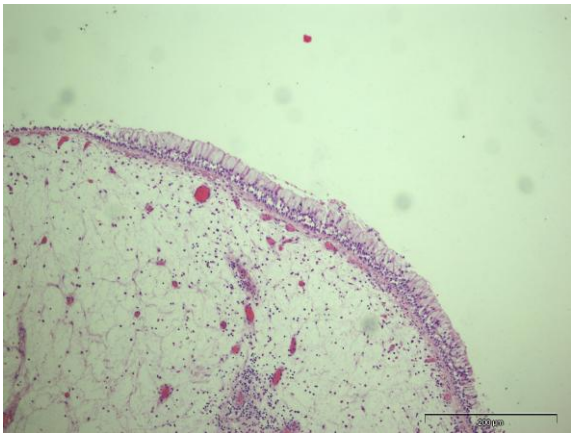

Fig. 3 – Nasal polyp with goblet cell metaplasia (hematoxillin & eosin, original magnification ×200).

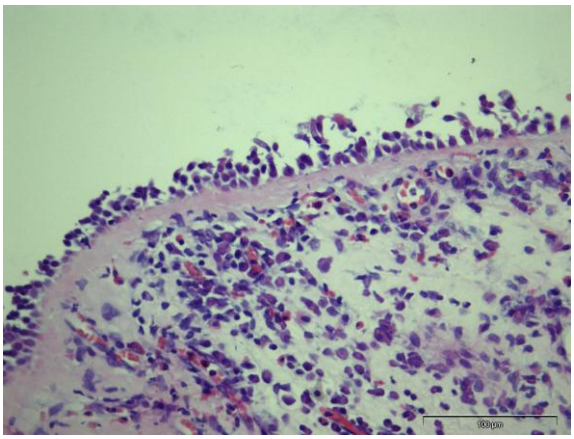

Fig. 4 – Basal membrane thickening within nasal polyp (hematoxillin & eosin, original magnification ×400).

Discussion

Rhinosinusitis can be defined as an inflammation with two or more of the following symptoms: nasal congestion/blockade, nasal discharge, facial pain, reduction/loss of smell; there are also complementary endoscopic signs and computed tomography changes. If rhinosinusitis persists for more than 12 weeks it is classified as chronic, with or without NP. NP consists of mucosal edema, inflammatory infiltrates, hyperplastic / hypertrophic sero-mucous glands often with some degree of epithelial metaplasia. A vast variety of inflammatory cells can be found in NP such as eosinophils, neutrophils, mast cells, plasma cells, lymphocytes, monocytes and fibroblasts. CRSwNP is also characterized with increased fibrosis and collagen deposition and with thickened epithelial basement membrane. Recent studies often discuss and explain different immunological pathways of tissue damage and edema, also different inflammatory pathways and different responses to treatment between CRSwNP and CRS without NP [10–12]. It is well known that inflammatory reactions can stimulate epithelial proliferation. Inflammatory cells produce various growth factors that stimulate epithelial proliferation. Recent studies report that NP with recurrent disease displayed higher scores for proliferation markers [12], but not significantly higher than that in non-recurring NP; preoperative steroid treatment might have resulted in inhibition of inflammatory response [12]. The presence of eosinophils greatly increases the risk of recurrent disease [13, 14]. Nakayama et al. [13] report eosinophilic inflammation in 59.6% of patients with NP. Patients with mucosal eosinophilia had higher recurrence rate than patients without mucosal eosinophilia, whereas patients with NP did not have higher polyp recurrence rate than patients without NP [13]. Vlaminck et al. [14] found tissue eosinophils in 78% of CRS with NP in comparison to 42% patients with CRS without NP. Eosinophilic mucin was observed in 52% of patients with CRSwNP and in 20% of patients CRS without NP. CRSwNP patients showed a recurrence rate of 48%; those with additional eosinophilic mucin showed 56% of recurrences [14]. In our study, after the follow-up period, there were no reccurences.

Recently macrophages invaded the spotlight in NP. Banks et al. [15] found that NP patients had significantly increased numbers of macrophages compared to control patients or patients without polyposis, regardless of atopic status. Our results concur with this report: we found significantly higher number of eosinophils, macrophages and plasma cells in patients with NP compared to the control ones, regardless of symptom duration, patients age and atopic status. We also found significant positive correlation between degree of fibrosis within NP and duration of symptoms and correlation between percentage of macrophages and degree of fibrosis. There was no such correlation between degree of tissue edema and age/duration of symptoms. We found that higher degree of tissue fibrosis may aggravate the operating process during endoscopic nasal surgery. We also found that younger patients with NP had significantly higher degree of neutrophils in inflammatory infitrates, regardless of symptom duration. These findings were not reported in previously published histopathological studies.

Conclusion

Tissue samples from patients with nasal polyposis show significant changes reflecting in various degrees of inflammation, fibrosis and basement membrane thickening which may contribute to more difficult surgical management and perioperative complications such as bleeding.

REFERENCES

1. *Prasad ML, Perez-Ordonez B.* Nonsquamous Lesions of the Nasal Cavity, Paranasal Sinuses, and Nasopharynx. In: *Gnepp DR*, editor. Diagnostic Surgical Pathology of the Head and Neck. Philadelphia: Saunders Elsevier; 2009; p. 112–4.
2. *Jankowski R.* Eosinophils in the pathophysiology of nasal polyps. Acta Otolaryngol (Stockh) 1996; 116(2): 160–3.
3. *Edward JA, Sanyal M, Le W, Soudry E, Ramakrishnan VR, Bravo DT*, et al. Selective expansion of human regulatory T cells in nasal polyps, and not in adjacent tissue microenvironments in individual patients exposed to steroids. Clin Immunol 2017; 179: 66–76.
4. *Petruson B, Hansson HA, Petruson K.* Insulin-like growth factor I is a possible pathogenic mechanism in nasal polyps. Acta Otolaryngol (Stockh) 1988; 106(1–2): 156–60.
5. *Alun-Jones T, Hill J, Leighton SE, Morissey MS.* Is routine histological examination of nasal polyps justified? Clin Otolaryngol Allied Sci 1990; 15(3): 217–9.
6. *Diamantopoulos II, Jones NS, Lowe J.* All nasal polyps need histological examination: an audit-based appraisal od clinical practice. J Laryngol Otol 2000; 114(10): 755–9.
7. *Kale SU, Mohite U, Rowlands D, Drake-Lee AB.* Clinical and histopathological correlation od nasal polyps: are there any surprises? Clin Otolaryngol Allied Sci 2001; 26(4): 321–3.
8. *Andrade GC, Fujise LH, Fernandes AM, Azoubel R.* Rhinosinusal Polyposis and Inverted Papilloma: A Morphometric Comparative Study. Int Arch Otorhinolaryngol 2015; 19(3): 196–9.
9. *Brescia G, Marioni G, Franchella S, Ramacciotti G, Giacomelli L, Marino F,*et al. A prospective investigation of predictive parameters for post-surgical recurrences in sinonasal polyposis. Eur Arch Otorhinolaryngol 2016; 273(3): 655–60.
10. *Hirschberg A, Kiss M, Kadocsa E, Polyanka H, Szabo K, Razga Z,* et al. Different activations of toll-like receptors and antimicrobial peptides in chronic rhinosinusitis with or without nasal polyposis. Eur Arch Otorhinolaryngol 2016; 273(7): 1779–88.
11. *Azizzadeh Delshad A, Jalali Nadoushan M, Davati A, Rostami A.* Expression of Vascular Endothelial Growth Factor in Nasal Polyp and Chronic Rhinosinusitis. Iran J Pathol 2016; 11(3): 231–7.
12. *Tezer I, Celebi Erdivanli O, Sanli A, Aydin S.* Could cellular proliferation be a predictive index for the relapse of nasal polyposis and down-regulated by nasal steroid treatment? Indian J Otolaryngol Head Neck Surg 2013; 65(Suppl 2): 329–32.
13. *Nakayama T, Yoshikawa M, Asaka D, Okushi T, Matsuwaki Y, Otori N,* et al. Mucosal eosinophilia and recurrence of nasal polyps – new classification of chronic rhinosinusitis. Rhinology 2011; 49(4): 392–6.

14. *Vlaminck S, Vauterin T, Hellings PW, Jorissen M, Acke F, Van Cauwenberge P,*et al. The importance of local eosinophilia in the surgical outcome of chronic rhinosinusitis: a 3-year prospective observational study. Am J Rhinol Allergy 2014; 28(3): 260–4.
15. *Banks CA, Schlosser RJ, Wang EW, Casey SE, Mulligan RM, Mulligan JK.* Macrophage Infiltrate Is Elevated in CRSwNP Sinonasal Tissue Regardless of Atopic Status. Otolaryngol Head Neck Surg 2014; 151(2): 215–20.

Received on December 28, 2017.
Revised on June 26, 2018.
Accepted on August 13, 2018.
Online First September, 2018.