Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the Vojnosanitetski Pregled. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article: NASAL POLYPOSIS: A SEMIQUANTITATIVE MORPHOMETRIC HISTOPATHOLOGICAL STUDY

NAZALNA POLIPOZA: SEMIKVANTITATIVNA MORFOMETRIJSKA HISTOPATOLOŠKA STUDIJA


UDC:

DOI: https://doi.org/10.2298/VSP171228139T

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
NASAL POLYPOSIS: A SEMIQUANTITATIVE MORPHOMETRIC HISTOPATHOLOGICAL STUDY

NAZALNA POLIPOZA: SEMIKVANTITATIVNA MORFOMETRIJSKA HISTOPATOLOŠKA STUDIJA

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

*Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical Centre of Serbia

† Department of otorhinolaryngology and maxillofacial surgery, Faculty of Medicine, University of Belgrade, Serbia

**Institute of Pathology, Faculty of Medicine, University of Belgrade, Serbia

§Institute of microbiology and immunology

¶Clinic for maxillofacial surgery Belgrade University School of dentistry

††Dental Clinic of the Military Medical Academy

Corresponding author:
Aleksandar Trivić
Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical Centre of Serbia, Pasterova 2
+381 63 235 757
Fax: +381 11 26 43 694
11000 Belgrade, Serbia
Mail to: drcole@sbb.rs
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.** Study was designed as prospective study with semiquantitative morphometric analysis and it comprised 77 patients with chronic rhinosinusitis and nasal polyposis that underwent functional endoscopic sinonasal surgery performed by the same surgeon. 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 study group showed significantly higher degree of inflammation than samples from control group ($\chi^2 = 35.89$ with $p$ value less than 0.01). Degree of fibrosis in nasal polyposis is 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 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 controls, with $p$ value less than 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 polyposis, semiquantitative morphometric analysis, histopathological hallmarks

Apstrakt

**Uvod / Cilj.** Nazalni polipi predstavljaju inflamatorne izrasline hipertrofične respiratorne sluznice i sačinjeni su od epitelnih i stromalnih elemenata. Cilj ove studije je da odredimo histopatološka obeležja nazalnih polipa kroz semikvantitativnu morfometrijsku studiju. **Metode.** Studija je dizajnirana kao prospektivna studija i obuhvata semikvantitivnu morfometrijsku analizu kod 77 pacijenata sa hroničnim rinosinuzitisom i nazalnim polipima. Kod svih pacijenata je učinjena funkcionalna endoskopska sinus hirurgija od strane istog hirurga. Morfometrijska analiza uključuje gradaciju edema tkiva sa polipima, debljinu bazalne membrane, stepen inflamacije, prisustvo/odsustvo metaplasije 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 ($\chi^2 = 35.89$ sa $p$ vrednosti manjom od 0.01). Stepen fibroze kod polipa nosa je u pozitivnoj korelaciji sa trajanjem dužine simtoma ($r = 0.25$, $p < 0.05$) i sa procentom makrofaga u zapaljenskom infiltratu ($r = 0.26$, $p < 0.05$). Pacijenti sa nazalnom polipozom imaju 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, sa $p$ vrednošću manjom od 0.01. **Zaključak.** Uzorci kod pacijenata 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 komplikacija kao što je krvarenje.

Ključne reči: nazalna polipoza, semikvantitativna morfometrijska analiza, histopatološka obeležja

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\(^1,2,3\); polyps are frequently associated with salicylates intolerance, asthma and cystic fibrosis\(^1,2,3,4,5,6,7,8,9\). Symptoms at presentation include nasal obstruction, rhinorrhea, headache, impaired sense of smell and postnasal discharge\(^1,2,3,4,5\). Nasal polyposis (NP) is slightly more prevalent in men, with an incidence in the fifth decade of life\(^5\), and affects between 1 and 4% of the population. 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 with nasal polyps (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 prospective study during period of January 1\(^\text{st}\) 2016 until December 31\(^\text{sr}\) 2016. 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 post-operatively. 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. 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.

Morphometric analysis was semiquantitative and included gradation of tissue edema within polyps according to A 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 control and 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 Chi-square test, Pearson's correlation coefficient and t-test with p values ≤0.05 that were considered significant. All analyzes were done in the software package Statistical Package for Social Sciences 18 (SPSS 18).

Results

Our study included 77 patients, 46 male (59.7%) and 31 female (40.3%). Control group consisted of 9 patients-6 were male (66.7%) and 3 female (33.3%). 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 had bilateral NP (89.6%). We found no gender differences in our patients in comparison with any of examined morphological data. Samples from study group showed significantly higher degree of inflammation than samples from control group (χ²=35.89 with p value less than 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 study group, whilst 17 showed no morphological signs of fibrosis. Degree of fibrosis in NP is 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 controls, with p value less than 0.01.
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 patient showed no adaptive epithelial changes (1.3%). We also found basal membrane thickening (Figure 4). doesn’t correlate with age and duration of symptoms.

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. CRS with NP 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 CRS with NP and CRS without NP. 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, but not significantly higher than that in non-recurring NP; preoperative steroid treatment might have resulted in inhibition of inflammatory response. The presence of eosinophils greatly increases the risk of recurrent disease. Nakayama et al. 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. Jorissen et al. 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 CRS with NP and 20% of patients CRS without NP. CRS with NP patients showed a recurrence rate of 48% ; those with additional eosinophilic mucin showed 56% of recurrences. In our study, after the follow up period, there were no recurrences.

Recently macrophages invaded the spotlight in NP. Banks et al. 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 control, 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 find 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 infiltrates, 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 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.

References


Table 1.

Clinical characteristics of the study group with nasal polyposis and the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Male Gender (%</th>
<th>Female Gender (%</th>
<th>Total</th>
<th>Age/y (min)</th>
<th>Age/y (max)</th>
<th>Duration of symptoms/m (min)</th>
<th>Duration of symptoms/m (max)</th>
<th>Average symptom duration/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6 (66.7)</td>
<td>3 (33.3)</td>
<td>9</td>
<td>18</td>
<td>55</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Study</td>
<td>46 (59.7)</td>
<td>31 (40.39)</td>
<td>77</td>
<td>13</td>
<td>71</td>
<td>31</td>
<td>12.10±6.81</td>
<td>(100)</td>
</tr>
</tbody>
</table>
Fig. 1– Histopathological hallmarks of the study group with nasal polyposis.

Figure 2. Nasal polyp with mild stromal fibrosis. H&E, original magnification x 200

Figure 3. Nasal polyp with goblet cell metaplasia. H&E, original magnification x 200

Figure 4. Basal membrane thickening within nasal polyp. H&E, original magnification x 400

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