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# Inducible nitric oxide synthase (iNOS) in sinonasal polyp pathogenesis

N. Bayar Muluk<sup>1</sup>, O. K. Arıkan<sup>2</sup>, P. Atasoy<sup>3</sup>, R. Kılıç<sup>1</sup> and E. Tuna Yalçinozan<sup>4</sup>

<sup>1</sup>ENT Department, <sup>3</sup>Pathology Department, Kırıkkale University, Faculty of Medicine, Kırıkkale, Turkey; <sup>2</sup>ENT Department, Adana Numune Research and Training Hospital, Adana, Turkey; <sup>4</sup>ENT Department, Artvin State Hospital, Artvin, Turkey

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Abstract. Inducible nitric oxide synthase (iNOS) in sinonasal polyp pathogenesis. Objectives: We investigated the role of inducible nitric oxide synthase (iNOS) in the pathogenesis of sinonasal polyps.

*Methods*: Adult patients (21 men, 3 women) with nasal polyposis underwent functional endoscopic sinus surgery. Nine adults without polyps (6 men) who underwent septoplasty and/or rhinoplasty served as controls. Polyp specimens came from three regions: the maxillary sinus (10), ethmoid sinus (14), and nasal cavity (10). Control group samples (9) came from the inferior turbinate. Specimens were evaluated in eight mucosal layers for count and distribution of inflammatory cells and iNOS expression. An iNOS positivity index (PI) was determined for the epithelium (E), subepithelial layer of the lamina propria (SE), and deep paraglandular layer of the mucosa (D).

*Results*: Polymorphonuclear cell (PMNC) % values of the ethmoid and maxillary sinus and overall ethmoid sinus PI were significantly higher in the polyp group. Patients with longer polyp duration, D-perivascular (D-pv), and a higher Brinkmann index had decreased ethmoid sinus D PIs. However, in older patients and patients with longer polyp duration, perivascular PIs increased in maxillary sinus SE and D, respectively. Furthermore, as PMNC % and iNOS-PMNC PI increased, SE\_glandular and epithelial\_apical iNOS values decreased. In the ethmoid and maxillary sinuses, iNOS\_D\_ endothelial values increased but decreased in the nasal cavity.

*Conclusions*: iNOS may play a role in sinonasal polyp pathogenesis, especially in mucosal SE and D layers. Increased vascular permeability, stromal edema, inflammatory cell migration into the stroma of the mucosa, and increased mucosal gland secretion may result in polyp formation.

#### Introduction

Inflammation of the upper airways is among the most common and frequent diseases and a category that includes nasal polyposis, associated in many cases with immune response disorders. An inflammatory infiltration of mononuclear, eosinophil, plasma, and mast cells can be found in the histological structure of the polypous as well as tonsillar mucosa.<sup>1</sup>

Several mechanisms have been proposed for the formation of nasal polyps, including allergy, mucosal allergy, autonomic imbalance, nitric oxide (NO), superantigens, infection, abnormal transepithelial ion transport, mucopolysaccharide abnormality, mechanical obstruction, and epithelial rupture. Eosinophils comprise more than 60% of the cell population. Activated T cells, mast cells, and plasma cells are also increased compared with the normal nasal mucosa. The stroma has numerous mediators, including cytokines, growth factors, adhesion molecules, and immunoglobulins. Both Th1 and Th2 types of cytokines are upregulated independent of the atopic status. Increased production of GM-CSF, IL5, RANTES, and eotaxin can contribute to chronic eosinophilic inflammation by regulating the migration, survival, and activation of eosinophils.<sup>2,3</sup>

NO is a ubiquitous biologically active radical produced in large quantities by healthy human paranasal sinus epithelium. NO (formula .N = O) is a simple, inorganic, gaseous free radical with predominant functions as a messenger and effector molecule. In mammals, NO is synthesized from L-arginine by a family of enzymes referred to as the nitric oxide synthases (NOS)<sup>4</sup> and is an important

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cellular signaling molecule with a vital role in many biological processes.<sup>5</sup> NO synthesis is catalyzed by one of three isoforms of NOS: neuronal NOS, endothelial NOS (eNOS), and inducible NOS (iNOS).<sup>6</sup>

Moderate immunostaining for iNOS has been identified in surface epithelium; glandular, inflammatory, and vascular endothelial cells; and smooth muscle cells in specimens from patients with chronic rhinitis only.<sup>47</sup> *In situ* hybridization shows eNOS mRNA expression in sites similar to those identified by immunohistochemistry whereas the mRNA for iNOS predominantly localizes to inflammatory cells.<sup>7</sup>

In the present study, we investigated the role of iNOS in the pathogenesis of nasal polyps. The polyp specimens were evaluated in eight layers of the mucosa for the count and distribution of inflammatory cells and their iNOS expression. Confounding factors affecting the iNOS positivity index (PI) levels at the epithelial, subepithelial, and deep layers were also investigated.

### Materials and methods

The study was performed in the Otolaryngology Department of Kirikkale University Faculty of Medicine. The immunohistochemical staining and light microscopy examinations were performed by the university's pathology department. All steps of the study were planned and conducted with approval from the Kirikkale University Faculty of Medicine Local Ethics Committee and according to the principles outlined in the Declaration of Helsinki.<sup>8</sup>

## Subjects

The nasal polyp group was selected from patients examined in the Otolaryngology Department of Kirikkale University Faculty of Medicine. They had used topical corticosteroid nasal spray for at least 6 weeks and until the day of surgery; if the pathology persisted, surgery was performed. This group consisted of 24 adults (21 men) with nasal polyps who underwent functional endoscopic sinus surgery (FESS). Patient ages were between  $23 \le$  and  $\le 70$ ; the mean age was  $46.5 \pm 11.2$ . All patients in the study group answered a questionnaire and underwent an ENT examination, endoscopic examination with 0° and 30° endoscopes, Waters' view radiography, and axial and coronal computed

tomography (CT) of the paranasal sinuses.

The control group consisted of nine adults without nasal polyps (6 men) who underwent a septoplasty. Their ages ranged between  $18 \le$  and  $\le 55$ ; the mean age was  $28.22 \pm 12.24$ . They agreed to enter the study and gave written approval, and all of the examination processes described above were also performed for the participants in the control group. In both groups, there were no other acute or chronic diseases, such as asthma, chronic obstructive pulmonary diseases, or acute or chronic sinusitis.

#### Methods

*Questionnaire*. The questionnaire covered anterior and posterior nasal discharge, nasal congestion, cough, facial and dental pain, halitosis, paroxysmal nocturnal coughing spells, sore throat, fever, olfactory loss, headache, and ear pain.<sup>9</sup> Polyp duration was assessed by hospital data and patient history, including the time of first diagnosis of a nasal polyp and follow-up data.

*Endoscopic examination*. Endoscopic examination with 0° and 30° endoscopes was performed in the Endoscopy Unit of the ENT Department of Kirikkale University Faculty of Medicine. Discharge (none, clear and thin, thick, purulent); mucosal status (normoplasia, light hyperplasia with no erythema, hyperplasia),<sup>10</sup> anatomic anomalies (septal deviation, lateral rotation of the uncinate process, turbinate hypertrophy, and other anatomic anomalies);<sup>9</sup> and localization and size of the polyps were examined.

In preoperative nasal endoscopic examination of the polyp group, appearance of nasal polyps was staged according to Lawson (1991).<sup>11</sup> Stage 0, no polyp presented; Stage 1, polyp under medial turbinate seen by endoscopy; Stage 2, protruding polyp in medial turbinate seen without endoscopy; and Stage 3: massive polyposis.

*CT*. Localization and size of polyps in the nasal cavity and paranasal sinuses were evaluated by axial and coronal sections of the paranasal sinuses in the polyp group. Also investigated were panpolyposis, septal deviation, concha bullosa, lateral rotation of the uncinate process, prominent ethmoid bulla, and other anatomic abnormalities.<sup>9</sup>

*Surgery*. All patients with polyps underwent FESS for nasal polyposis. Biopsies were performed under general anesthesia with samples obtained from macroscopically observed polypoid areas.

Specimens including polyp tissue were excised from three regions: the nasal cavity and the maxillary and ethmoid (anterior and posterior) sinuses. The specimens were examined at x400 magnification under light microscopy, and only slides with histopathological evidence of polypoid tissue were included in the study. Tissues that were edematous and rich in vessels, with inflammatory cells and polypoid development, were included in the study as the polyp group. Slides suggesting a chronic inflammatory process without polypoid tissue were excluded. Finally, the study group covered three regions: ethmoid sinuses (14 specimens), maxillary sinus (10 specimens), and nasal cavity (10 specimens). In the control group, specimens were collected via punch biopsies from inferior turbinates during septoplasty for a total of nine specimens in the control group.

*Immunohistochemical staining*. In the study and control groups, a surgical specimen was examined with an immunohistochemical staining technique using a monoclonal antibody against iNOS, nitric oxide synthase, inducible Ab-1 rabbit polyclonal antibody (Thermo Scientific/LabVision Corporation, Fremont, CA, USA). In each of the surgical specimens, iNOS positivity was evaluated in 3–4 high-magnification fields under light microscopy and the mean number of cells in the epithelium (E), subepithelial layer of the lamina propria (SE), and deep paraglandular layer of the mucosa (D) determined.

Immunohistochemical staining technique. Sections in 5  $\mu$ m thickness were obtained, transferred to adhesive slides, and dried in an autoclave at 37°C overnight and at 60°C for 20 minutes. They were deparaffinized and dehydrated by immersion in xylene twice for 10 minutes and in alcohol twice for 10 minutes. The specimen was then incubated in 3% H<sub>2</sub>O<sub>2</sub> for 5 minutes to inhibit endogenous peroxidases. The preparations were transferred into a citrate-based antigen retrieval solution (Dako; Denmark; pH 6) for iNOS antigens (Thermo Scientific/ LabVision Corporation Neomarkers, Fremont, CA, USA). The nitric oxide synthase, inducible Ab-1 rabbit polyclonal antibody (Thermo Scientific/Lab-Vision Corporation, Fremont, CA, USA). was used. All slides were microwaved (750 W) for 5 minutes two times. Following the instructions in the Shandon Sequenza manual for standardization, the classical streptavidin-avidin/biotin-peroxidase method and diaminobenzidine chromogen (20 minutes)

were applied for immunohistochemical analysis of antibody binding. Non-immune mouse serum served as a negative control, and Mayer's hematoxylin was used as counterstain. Cytoplasmic staining was considered evidence of positivity.

An expert pathologist reviewed the slides. In each slide, to evaluate the inflammatory cells, initially hematoxylin-eosin (H-E) sections were prepared and examined under light microscopy. For each H-E section, we identified inflammatory cells: polymorphonuclear cells (PMNCs), including polymorphonuclear leukocytes and eosinophils; mononuclear cells (MNCs), including lymphocytes, plasma cells, mast cells, and histiocytes; and fibroblasts. Then iNOS expression and number of inflammatory cells were determined under light microscopy (Leica, Germany) per 100 cells in 3-4 high-magnification fields. Means of cell counts were calculated as % values, and iNOS PI values for inflammatory cells were evaluated on a scale of 0 to 3: PI 0, antigen (iNOS)+ cell count was 0% (no stained cells); PI 1, antigen (iNOS)+ cell count was <5%; PI 2, antigen (iNOS)+ cell count was 5–50%; and PI 3, antigen (iNOS)+ cell count was >50%.

*PI*. For the quantitative assessment of iNOS expression, staining in the E, SE, and D and inflammatory cells was assessed by counting 100 cells in 3–4 high-magnification fields and calculating means. Means for the iNOS (+) cells per 100 cells on a high-magnification field (×400) were derived in the E, SE, and D. Scoring was performed on a 0–3 scale, in which 0 represented negative staining; 1, weakly positive; 2, positive; and 3, strongly positive.<sup>12</sup> For the PI, values indicated the following: PI 0, antigen (iNOS)+ cell count was 0% (no stained cells); PI 1, antigen (iNOS)+ cell count was 5–50%; and PI 3, antigen (iNOS)+ cell count was >50%.

Assessment was performed at eight levels: (1) Epithelial\_apical (EP\_apical), (2) Epithelial\_basal (EP\_basal), (3) Subepithelial\_perivascular (SE\_pv), (4) Subepithelial\_glandular (SE\_gland), (5) Subepithelial\_ endothelial (SE\_endothelial), (6) Deep\_ perivascular (D\_pv), (7) Deep\_glandular (D\_ gland), and (8) Deep\_Endothelial (D\_endothelial).

#### Statistical analysis

The statistical packet for SPSS (version 16.0) was used for statistical evaluation. For the four groups – nasal cavity, maxillary sinus, ethmoid sinus, and control – the differences for iNOS PI were analyzed using Kruskal-Wallis variance analysis. When a statistically significant result was found, pairwise comparisons were made using the Mann-Whitney U Test with Bonferroni correction to detect the group that caused the difference.

In the study group, correlations between age, sex, polyp duration, smoking, and Brinkmann index values and iNOS levels in the ethmoid sinus, maxillary sinus, and nasal cavity were respectively analyzed separately using Spearman's correlation rho efficient in the three polyp groups.

The confounding factors (covariates: age, sex, polyp duration, smoking, and Brinkmann index, PMNC-%, MNC-%, iNOS-PMNC, iNOS-MNC, iNOS-all cells) affecting iNOS-PI in mucosal layers (E, SE, and D) were analyzed using linear regression analysis (backward, step-by-step analysis) in the three polyp groups. A p value <0.05 was considered statistically significant.

#### Results

Table 1 and Figures 1-3 illustrate medians of iNOS PI values in the EP\_apical, EP\_basal, SE\_pv, SE\_gland, SE\_endothelial, D\_pv, D\_gland, and D\_endothelial parts of the ethmoid sinus, maxillary

sinus, and nasal cavity and control group samples, and for MNC-% and iNOS-PI, PMNC-%, and iNOS-PI, and all-cell iNOS-PI.

For iNOS\_D\_gland PI, the four groups differed significantly (p = 0.043), but no statistically significant difference was found for other iNOS PI values of the mucosal layers (p>0.05) (Table 1). To identify the group responsible for the significant difference in the iNOS\_D\_gland PI, pairwise comparisons were performed with Bonferroni correction, but no significant difference was detected (p>0.0125) (In Bonferroni adjustment process, adjusted alpha value was used. alpha<sub>adjusted</sub> value = 0.0125).

A statistically significant difference was found for PMNC-% (p = 0.011) and iNOS-all\_cells (p = 0.043) among the four groups (Table 1). To identify the group responsible for the difference, pairwise comparisons were performed with Bonferroni correction and showed that PMNC-% values for the ethmoid sinus (median 25.0) (p = 0.005) and maxillary sinus (median 55.0) (p = 0.006) were significantly higher than for the control group (median 5.0) (Figure 2). In addition, the iNOS-PI of all cells of the ethmoid sinus (median 2.0) was significantly higher than that of the control group (median 1.0) (p = 0.010) (Figure 3).

	Groups				
iNOS positivity index	Ethmoid sinus $(n = 14)$	Maxillary sinus (n = 10)	Nasal cavity (n = 10)	Control (n = 9)	p*
iNOS_EP_apical	2.0	2.0	2.0	2.0	0.502
iNOS_EP_basal	2.0	2.0	2.0	2.0	0.116
iNOS_SE_pv	1.0	1.5	1.0	1.0	0.480
iNOS_SE_gland	2.0	2.0	2.0	2.0	0.223
iNOS_SE_endothelial	1.0	1.0	0.5	1.0	0.434
iNOS_D_pv	1.0	1.0	1.0	1.0	0.631
iNOS_D_gland	1.0	1.5	0.5	2.0	0.043
iNOS_D_endothelial	1.0	1.0	0.0	0.0	0.055
Inflammatory cells					
PMNC-%	25.0	25.0	22.5	5.0	0.011
iNOS-PMNC	0.5		0.0	0.0	0.463
MNC-%	75.0		77.5	90.0	0.423
iNOS-MNC	2.0		2.0	2.0	0.228
iNOS-all_cells	2.0		2.0	1.0	0.043

*Table 1* iNOS positivity index levels in ethmoid sinus, maxillary sinus, nasal cavity, and control groups (medians)

\*p value shows the results of Kruskal-Wallis variance analysis.

#### iNOS in sinonasal polyp pathogenesis





*Figure 1* iNOS positivity index levels in mucosa of groups 1–4

In the polyp group, correlations between age, sex, polyp duration, smoking, and Brinkmann index values and iNOS levels in the ethmoid sinus, maxillary sinus, and nasal cavity were analyzed separately. For the ethmoid sinus, as polyp duration increased, iNOS\_D\_pv decreased (p = 0.017, r = -0.727). In patients with a higher Brinkmann index, iNOS\_D\_endothelial decreased (p = 0.037, r = -0.633). For the maxillary sinus, as age increased, iNOS\_SE\_pv also increased (p = 0.027, r = 0.691), and iNOS\_D\_pv increased with longer polyp duration (p = 0.028, r = 0.808).

The confounding factors (covariates: age, sex, polyp duration, smoking, and Brinkmann index, PMNC-%, MNC-%, iNOS-PMNC, iNOS-MNC, iNOS-all cells) affecting iNOS-PI in the layers of the mucosa (epithelial, subepithelial and deep) were analyzed by linear regression analysis (Figure 4). For iNOS\_EP\_apical, as iNOS-PMNC PI increased, iNOS\_EP\_apical decreased (p = 0.038, r = -426) while for the iNOS\_SE\_gland, as PMNC-% increased, iNOS\_SE\_gland value decreased

(p = 0.017, beta = -6.602), as it also did with increasing MNC-% (p = 0.020, beta = -6.348). iNOS\_D\_pv, on the other hand, was increased in smoking patients (p = 0.032, r = 0.745) but decreased in patients with a higher Brinkmann index (p = 0.012, r = -0.896). Finally, in the ethmoid and maxillary sinuses, iNOS\_D\_endothelial values increased relative to nasal cavity; whereas they decreased in the nasal cavity relative to ethmoid and maxillary sinuses (Median values of iNOS\_D\_endothelial values were 1.0, 1.0 and 0.0 in ethmoid sinus, maxillary sinus and nasal cavity polyps respectively) (p = 0.014, r = -0.532) (Figure 4).

### Histopathologic findings

Light microscopy examination revealed that pseudostratified ciliated epithelium was present in the majority of polyps, and a very small portion of the polyps were also lined with metaplastic epithelium. In iNOS-stained sections, strong positivity was specifically detected in the apical portion of the



*Figure 2* Distribution of inflammatory cells in the mucosa.

Based on the Mann–Whitney U test with Bonferroni correction, PMNC-% values of ethmoid sinus (median 25.0) (p = 0.005) and maxillary sinus (median 55.0) (p = 0.006) were significantly higher than that of the control group (median 5.0).



*Figure 3* iNOS PI levels in inflammatory cells.

Based on the Mann–Whitney U test with Bonferroni correction, iNOS-PI of all cells of ethmoid sinus (median 2.0) was significantly higher than that of the control group (median 1.0) (p = 0.010).



Figure 4

Coefficient (beta) values and covariates as significantly detected confounding factors on iNOS PI, as detected by linear regression analysis (backward).

(-) Coefficient (beta) values are presented. Covariates were age, sex, polyp duration, smoking, and Brinkmann index, PMNC-%, MNC-%, iNOS-PMNC, iNOS-MNC, and iNOS-all cells. When the iNOS-PMNC PI increased, iNOS\_EP\_apical decreased (p = 0.038, r = -426); as PMNC-% increased, iNOS\_SE\_gland value decreased (p = 0.017, beta = -6.602); as MNC-% increased, iNOS\_SE\_gland value decreased (p = 0.017, beta = -6.602); as MNC-% increased, iNOS\_SE\_gland value decreased (p = 0.017, beta = -6.602); as MNC-% increased, iNOS\_SE\_gland value decreased (p = 0.017, beta = -6.002); as MNC-% increased, iNOS\_SE\_gland value decreased (p = 0.017, beta = -6.002); as MNC-% increased, iNOS\_SE\_gland value decreased (p = 0.017, beta = -6.002); as MNC-% increased, iNOS\_D\_pv value decreased (p = 0.012, r = 0.745); in patients with a higher Brinkmann index, iNOS\_D\_pv value decreased compared to patients with lower Brinkmann Index (p = 0.012, r = -0.896); and in ethmoid and maxillary sinuses, iNOS\_D\_endothelial values increased whereas in the nasal cavity, iNOS\_D\_endothelial values decreased (p = 0.014, r = -0.532).

surface epithelium. In subepithelial areas, both the mucosal gland epithelium and endothelium showed diffuse immunostaining with iNOS. Although MNCs and fibroblasts showed prominent iNOS expression, except for a slight expression in the ethmoid sinus, almost no expression could be detected in polymorphonuclear leukocytes. In edematous areas of polyps, expression of all markers was less than in other areas (Figure 5).

#### Discussion

Nasal polyps is a multifactorial disease with infectious, noninfectious, inflammatory, anatomic, and



#### Figure 5

Immunohistochemical staining for iNOS in a polyp sample displaying expression in both the epithelial apical and basal layers. In the subepithelial layer, diffuse positive staining was observed in MNCs, vascular endothelial cells, and glands ( $\times 100$ ).

genetic abnormalities. Chronic inflammation remains the central major factor in nasal polyps.<sup>2</sup> Endogenously produced anti-microbial peptides like beta-defensins and molecules like NO are important components of the immune response. Disturbance of this response can have damaging consequences. Nasal polyposis, a common disorder, is an example of such immune dysregulation.<sup>13</sup>

NO concentrations in healthy sinuses can rise close to the highest permissible environmental pollution levels, leading to the hypothesis that NO contributes to sinus host defense by virtue of its antimicrobial properties and ability to upregulate ciliary activity.<sup>14</sup> The most important source of NO in the respiratory tract is iNOS. In the upper and lower airways, iNOS is primarily expressed in epithelial cells and macrophages and is upregulated by cytokines, microbes, or microbial products, permitting NO production to increase markedly in response to infection and in inflammatory states.<sup>14</sup>

Lorente *et al.*<sup>15</sup> reported that nasal polyps contain higher levels of total NO synthase activity than nasal mucosa tissue. In addition, nasal polyps exhibit mainly iNOS activity whereas all NOS activity detected in the nasal mucosa is in constitutive form. In both cases, NOS activity localizes in epithelial cells. In view of these findings, we inferred that NO may be an important inflammatory mediator in the respiratory system and that the epithelium may be a source of NO production in the upper airways.<sup>16</sup>

In the present study, we investigated the role of iNOS in the pathogenesis of nasal polyps. No statistically significant difference for iNOS PI values was found in the mucosal layers among the four groups. iNOS positivity was decreased from the SE to D layers because of stromal edema and reduced cell density. In other words, in edematous areas of polyps, expression of all markers was less than in other areas, which we attribute to the small number of cells in the edematous areas. This condition may explain the lack of difference in results of the statistical analysis of the four groups.

Strong eNOS immunoreactivity and weak iNOS immunoreactivity have been found in the columnar epithelium of human nasal mucosa in normal subjects.<sup>17</sup> In our study, however, intense iNOS expression was detected at the apical surface of the epithelial layer in all groups. Also, in our study, the percentage of PMNCs in the ethmoid and maxillary sinuses and iNOS-PI values of all cells of the ethmoid sinus were significantly higher compared to the control group. NO levels may reflect the inflammatory status of both the upper and lower airways. Because NO synthase is induced in inflamed upper airways,<sup>16</sup> an increase in inflammatory cells expressing iNOS in the ethmoid and maxillary sinuses was thought to play a role in the pathogenesis of sinonasal polyps in our study.

In addition, we observed that iNOS was not expressed in PMNCs except for very low expression in the ethmoid sinus. In the all-cells group of inflammatory cells, there were PMNCs, MNCs, and fibroblasts. Higher iNOS expression in the all-cells group compared to MNCs was the result of fibroblasts and their higher iNOS expression. Thus, iNOS expression in the all-cells group was mainly due to fibroblastic iNOS expression.

In addition to a role in nonspecific immunoreactions and inflammation in a variety of tissues, NO can play an important role in the regulation of vascular tone and neurotransmission. Many kinds of cells, such as macrophages, neutrophils, and endothelial and smooth muscle cells can express iNOS. Because all isoforms of NOS seem to be present in nasal tissues and the expression of iNOS under inflammatory conditions seems to be responsible for excessive production of NO, the distribution of NOS isoforms (especially iNOS) in normal and inflammatory nasal tissue, as well as the exact requirements for expression of iNOS, remains to be shown.<sup>18</sup> In the present study, at least, in patients with longer polyp duration and D-pv and in patients with a higher Brinkmann index value, D-endothelial iNOS PIs in ethmoid sinus and D-pv iNOS expression decreased. However, in maxillary sinus in older patients and in patients with longer polyp duration, perivascular iNOS PIs increased in the subepithelial and deep layers of the mucosa, respectively. Lower iNOS-endothelial PI levels may affect the vascular structure of the polyps, lessening circulation and resulting in more fibrotic polyps. Longterm and excessive smoking were also related to lower iNOS values at the perivascular region, causing a reduction in vascular permeability and less migration and extravasation of inflammatory cells to the mucosal stroma. Ultimately, the inflammatory reaction lessens and fibrotic polyps emerge. In older patients with longer polyp duration, iNOSperivascular PI values increased in the deep layer of the mucosa, causing increased vascular permeability and stromal edema.

Hess et al.,18 working with non-inflamed fresh human nasal mucosa from the middle turbinate, compared these samples immunohistologically with nasal mucosa manifesting the typical findings of chronic polypoid rhinosinusitis (i.e., polypoid middle turbinates and polyps of the middle nasal duct). Clearly increased or initiated expression of iNOS was detected in blood vessels, glands, macrophages, and epithelium of chronically inflamed and bacterial lipopolysaccharide-incubated nasal tissue in comparison to the non-inflamed controls.<sup>18</sup> In our study, increased PMNC percentages and iNOS PI values were related to decreased iNOS expression in the EP\_apical and SE\_glandular portions. Increased MNC percentages were also seen with lower iNOS expression in the SE glandular area. The results suggest that the increase in both PMNCs and MNCs was related to a decrease in iNOS glandular PI values and that fibroblasts, the other cells of the all-cells group (PMNCs+MNCs+fibrobasts) may be the main source of iNOS values in polyp tissues. Tewfik et al.<sup>19</sup> reported that NO stimulates collagen expression in human nasal polyp-derived fibroblasts. This stimulation appeared to favor the upregulation of collagen type III, leading to a shift in the ratio of collagen type I to type III production.<sup>19</sup>

In the deep endothelial parts of our polyp tissue samples, we observed increased iNOS expression in the ethmoid and maxillary sinuses and decreased iNOS expression in the nasal cavity. iNOS is induced by low oxygen tension.<sup>20</sup> At low tissue oxygen levels in nasal polyps, iNOS values increase in endothelial cells, causing increased vascular permeability. Vasodilatation and inflammatory cell migration may initiate the process of polypoid degeneration and edema in the stroma. Liu *et al.*<sup>21</sup> examined the expression and distribution of hypoxia-inducible factor-1alpha in nasal polyps and concluded that hypoxia may play an important role in the pathogenetic mechanism of nasal polyps.

In our study, iNOS expression was detected in both polyp tissues and the inferior turbinate samples of the control group, although with a significant difference among the four groups. In polyp tissue, because of edema in the subepithelial and deep layers of the mucosa, the cellular density was not as intense in these areas compared to controls. As noted, iNOS expression occurs in many kinds of cells,<sup>22</sup> which may explain the lack of differences among iNOS expression values. Watanabe and Kakuta<sup>23</sup> studied iNOS expression under light and electron microscopy, and immunoreactivity for iNOS was reported to be localized to the mucosal epithelium, inflammatory cells, vascular endothelium, smooth muscle, and nasal gland.<sup>23</sup>

Like Kirtsreesakul,<sup>2</sup> we suggest that chronic inflammation remains the central major factor in nasal polyps. Fundová et al.,24 investigating the expression of insulin-like growth factor-1 receptor (IGF-1R) and iNOS in nasal polyps compared to healthy nasal mucosa in both the epithelial and stromal compartments, detected positivity for iNOS within the epithelium of nasal polyps compared with the nasal mucosa. The numbers of iNOS-positive single cells were highly increased in nasal polyp vs. nasal mucosa in both epithelial (3.83 vs. 1.08) and stromal (4.96 vs. 2.67) compartments. An increased iNOS expression within the epithelial layer as well as increased number of iNOS- and IGF-1R-positive cells in nasal polyps also was observed. Their results suggest that the innate immune mechanism and to a lesser extent growth and homeostasis of epithelial cells may play a role in formation of nasal polyps. Chen and Xiang<sup>25</sup> also reported that iNOS was expressed mostly in the inflammatory cells inside the tissue; a large amount of NO produced by iNOS may lessen the inflammation process of nasal polyps.

The cellular expression of NOS in the human nasal mucosa suggests a possible role for iNOS in

the regulation of blood flow, nasal secretion, and ciliary movement in health and disease.<sup>7</sup> In our study, we inferred that iNOS may potentially play a role in sinonasal polyp pathogenesis through the vascular system, endothelial cells, and glandular cells, especially in the subepithelial and deep layers of the mucosa. Increased vascular permeability, stromal edema, inflammatory cell migration into the stroma of the mucosa, and increased secretion by mucosal glands may result in polyp formation.

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Dr. Nuray Bayar Muluk

Birlik Mahallesi, Zirvekent 2. Etap Sitesi, C-3 blok, No: 62/43 06610 Çankaya, Ankara, Turkey

Tel.: +90 312 4964073, +90 532 7182441

Fax: +90 318 2252819

E-mail: nurayb@hotmail.com

nbayarmuluk@yahoo.com