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The role of endothelial nitric oxide synthase (eNOS) in the pathogenesis of sinonasal polyps

N. BAYAR MULUK, O.K. ARIKAN[1], P. ATASOY[2], R. KILIÇ, E. TUNA YALÇINOZAN[3]

[1]ENT Department, Adana Numune Research and Training Hospital, Adana, Turkey
[2]Pathology Department; Faculty of Medicine, Kirikkale University, Kirikkale, Turkey
[3]ENT Department, Artvin State Hospital, Artvin, Turkey

Abstract. – OBJECTIVE: The pathogenesis of sinonasal polyps has not been known completely. We investigated the role of endothelial Nitric Oxide Synthase (eNOS) in the pathogenesis of sinonasal polyps.

PATIENTS AND METHODS: Study group (Groups 1-3) consisted of nasal polyp samples of patients with sinonasal polyps; and control group consisted of inferior turbinate samples of patients without nasal polyp. In Group 1: 14 specimens from ethmoid sinus; in Group 2: 10 specimens from nasal cavity; in Group 3: 10 specimens from maxillary sinus; and in Group 4 (Control): 9 specimens from inferior turbinate were included. By immunohistochemical staining technique, eNOS Positivity Index in mucosal layers; and in the inflammatory cells were assessed.

RESULTS: eNOS Positivity Index was higher at apical layer of epithelium; and perivascular and glandular parts of subepithelial layer. As a rate of mononuclear cells increased, eNOS positivity increased at basal part of epithelium. In eNOS Positivity Index of mononuclear cells increased ones, eNOS values also increased at glands of subepithelial layer. In nasal cavity, eNOS positivity index of all cells was significantly higher than that of the control group. Increased eNOS all cells positivity index values were seen with decreased glandular and endothelial eNOS values. In all cells group, fibroblasts were seen beside the mononuclear cells. It was observed that eNOS was not expressed in PMNC (mainly neutrophils), growing more in acute inflammatory process; and was expressed in MNCs and all cells group with fibroblasts which were the cells of chronic inflammatory process. Especially MNCs and fibroblasts may play a role in the polyp formation process. In males and in patients with longer polyp duration, eNOS values decreased.

CONCLUSIONS: We concluded that eNOS Positivity Index was higher at apical layer of epithelium; and perivascular and glandular parts of subepithelial layer. eNOS plays role in vascular dilatation, increases in vascular permeability; increases in nasal secretion due to glandular secretion; and edema in subepithelial and deep layers of the mucosa by affecting glands. Irritant agents in the breathing air and environment may cause increase in eNOS values at apical part of epithelium and may promote polyp formation by vasodilatation and increased glandular secretion due to increased nitric oxide values. In elderly patients and in long standing polyps, eNOS values decreased causing more fibrotic polyps.

Key Words:
Sinonasal polyp, Pathogenesis, Endothelial nitric oxide synthase (eNOS), Endothelial, Glandular, eNOS positivity index.

Introduction

The nasal mucosa plays an important role in defense of the lung against harmful agents. It has been suggested that this is partly mediated by the production of nitric oxide (NO)[1]. NO is a simple, inorganic, gaseous free radical whose predominant functions are that of a messenger and effector molecule. In mammals, NO is synthesised by a family of enzymes referred to as the nitric oxide synthases (NOSs)[2], a family of eukaryotic enzymes that catalyze the production of NO from L-arginine[1]. NO is an important cellular signaling molecule, having a vital role in many biological processes[3]. NO formation was first described in endothelial cells[4], and was subsequently reported in many cell types, such as neurons, fibroblasts, platelets, macrophages, neutrophils and epithelial cells[2,5,6].

First assays of NOS were performed in brains, vascular endothelium and macrophages[7,8]. NO synthesis is catalyzed by one of three isoforms of NOS: NOS I (neuronal or nNOS), NOS III (endothelial or eNOS), and NOS II (inducible or iNOS)[9]. eNOS has been demonstrated in endothelial cells of arteries[10], and veins[11]. eNOS-derived

Corresponding Author: Nuray Bayar Muluk, MD; e-mail: nurayb@hotmail.com

NO plays an important role in the regulation of microcirculation[12], and in the control of vascular smooth muscle cell tone, hence its alternative name endothelium derived relaxing factor (EDRF) first suggested by Palmer et al[13].

Nasal vasculature and seromucous glands are exposed to complex mechanisms influenced by external as well as internal stimuli. In addition to classic and peptidergic neurotransmitters, NO was increasingly found to be important in the control of various physiological functions. NO regulates nasal immunology, influences macrophages activity and has antiviral and bacteriostatic properties. The aim of this study was to detect the localization of nitric oxide synthases (NOS) I and III in the normal human nasal mucosa with immunoelectron microscopical techniques. Nitric oxide in nerval fibers, seromucous glands and endothelial cells of capillaries and arterial vessels suggests that NO takes part in the regulation of physiological processes of the human nasal mucosa[14].

In Bugdayci and Kaymakci's study[15], the pathophysiological role of NO and malondialdehyde (MDA) in tissue of patients with nasal polyposis were investigated. They reported that the level of NO_2 and NO_3 in tissue was higher than normal tissue ($p < 0.05$).

In the present study, we investigated the role of eNOS in the pathogenesis of nasal polyps. The polyp specimens were evaluated at eight layers of the mucosa. We also analyzed the confounding factors, most affecting the eNOS Positivity Index levels at epithelial, subepithelial and deep layers of the mucosa.

Patients and Methods

The study was assessed in the Ear Nose Throat (ENT) Department of Kirikkale University Faculty of Medicine. The immunohistochemical staining and light microscopic examination was performed by Pathology Department of Kirikkale University Faculty of Medicine.

Subjects

The nasal polyp group was selected from the patients examined in the Otolaryngology Department of Kirikkale University Faculty of Medicine. They have used topical corticosteroid nasal spray for at least 6-week duration; and if the pathology was going on, operation was performed. This study group consisted of 24 adult patients (21 males, 3 females) with nasal polyp who underwent Functional Endoscopic Sinus Surgery (FESS). Patient ages were between 23 and 70; and the mean age was 46.5 ± 11.2. The control group consisted of 9 adult patients without nasal polyp (6 males and 3 females) who underwent septoplasty operation. Their ages ranged between 18 and 55; and the mean age was 28.22 ± 12.24. They accepted to enter the study with written approval.

Study group (Groups 1-3) consisted of nasal polyp samples of patients with sinonasal polyps; and control group consisted of inferior turbinate samples of patients without nasal polyp. In Group 1: 14 specimens from ethmoid sinus; in Group 2: 10 specimens from nasal cavity; in Group 3: 10 specimens from maxillary sinus; and in Group 4 (Control): 9 specimens from inferior turbinate were included.

There were not any other diseases in subjects of both groups.

Method

All patients in the study underwent ENT examination and endoscopic examination with 0º and 30º endoscopes; and axial and coronal CT of paranasal sinuses were taken.

Endoscopic Examination

Endoscopic examination was performed with 0º and 30º endoscopes. Discharge (none, clear and thin, thick, purulent); mucosal status (normoplasia, light hyperplasia with no erythema, hyperplasia)[16]; anatomic anomalies (septal deviation, lateral rotation of the uncinate process, turbinate hypertrophy and other anatomic anomalies)[16]; and localization and size of the polyps were examined.

In preoperative nasal endoscopic examination of study group, appearance of nasal polyps was staged as Lawson's (1991)[17]. Stage 0: No polyp presented, Stage 1: Polyp under medial turbinate that was seen by endoscopy, Stage 2: Protruding polyp in medial turbinate that was seen without using endoscopy, Stage 3: Massive polyposis.

Computed Tomography

By axial and coronal sections of paranasal sinuses, in nasal polyp group, localization and size of the polyps in the nasal cavity and paranasal sinuses were evaluated. And also pan-polyposis, septal deviation, concha bullosa, lateral rotation of the uncinate process, prominent ethmoid bulla and other anatomic abnormalities[16] were also in-

vestigated. In control group, both coronal and axial computed tomographic evaluation were also assessed.

Operation

All patients of the study group underwent FESS for nasal polyposis. Biopsies were performed under general anesthesia. Samples were obtained from macroscopically observed polypoid areas. Specimens including polyp tissue were excised from 3 regions: nasal cavity, maxillary and ethmoid (anterior and posterior) sinuses. The specimens were examined at x 400 magnification under light microscopy; and the slides with polypoid tissue were included in the study. The tissues, which were edematous and rich in vessels; had severe inflammatory cells; and showed polypoid development, were included into the study as the study group. Slides including chronic inflammatory process without polypoid tissue were excluded. Finally, the study group consisted of three regions: ethmoid sinuses (including 14 specimens), maxillary sinus (including 10 specimens), nasal cavity (including 10 specimens).

In control group's subjects, specimens were collected via punch biopsies from inferior turbinates during septoplasty operation and 9 specimens were included into the control group.

Immunohistochemical Staining

In the study and control groups, surgical specimen was examined with immunohistochemical staining technique with monoclonal antibodies against eNOS. In each of the surgical specimens, the number of eNOS positivity were evaluated in 3-4 high magnification field on light microscope and the mean number of these cells in the epithelium (E), subepithelial layer of lamina propria (SE) and deep paraglandular layer of the mucosa (D) were determined.

Immunohistochemical Staining Technique

Five-micron-thick sections were obtained, transferred on to adhesive slides, and dried in autoclave at 37°C overnight and at 60°C for 20 minutes. They were deparaffinized and dehydrated by immersion into xylene twice for ten minutes and in alcohol twice for ten minutes. The specimen was then incubated in 3% H_2O_2 for five minutes to inhibit endogenous peroxidases. The preparations were transferred into citrate-based antigen retrieval solution (Dako, Glostrip, Denmark; pH: 6) for eNOS antigens (Lab Vision Corporation Neomarkers, Fremont, CA, USA). All slides were kept in microwave oven (750 watts) twice for five minutes. By using Shandon Sequeza Tm manual staining device for standardization, classical Streptavidine Avidin-Biotin-Peroxidase (Strept. AB-Peroxidase) method and diaminobenzidine (DAB) chromogen (20 minutes) were applied for immunohistochemical analysis of three antibodies. Non-immune mouse serum was served as a negative control and Mayer's haematoxylin was used as counterstain. Cytoplasmic staining was considered evidence of positivity[18].

The slides were reviewed by an expert pathologist. In each slide, the number of eNOS+ cells-inflammatory cells [Polymorpho nuclear cells (PMNCs), including polymorpho nuclear leukocytes and eosinophils; Mononuclear cells (MNCs) including lymphocytes, plasma cells, mast cells and histiocytes; and in all cells group, there were fibroblasts additionally PMNCs and MNCs] were counted by light microscope (Leica, Germany) per 100 cells in 3-4 high magnification field. Means of cell counts were calculated as % (or rate) values; and eNOS Positivity Index values at inflammatory cells were also detected on 0-3 scale.

Positivity Index (PI)

For the quantitative assessment of eNOS expression, staining in the epithelium (E), SE and Deep layers of the lamina propria; and inflammatory cells were assessed by counting totally 100 cells in 3-4 high magnification field and means were calculated. Eventually means of the eNOS (+) cells per 100 cells on a high magnification field (× 400) were detected in the E, SE and Deep layers of the lamina propria. Scoring was performed on a 0-3 scale, where 0 represented negative staining; 1, weakly positive; 2, positive; and 3, strongly positive[18]:

- PI 0 means that antigen (eNOS)+ cell count was 0% (No stained cells)
- PI 1 means that antigen (eNOS)+ cell count was < 5%,
- PI 2 means that antigen (eNOS)+ cell count was 5-50%,
- PI 3 means that antigen (eNOS)+ 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), (8) Deep_Endothelial (D_endothelial).

Statistical Analysis

Statistical packet for SPSS (Version 16.0, SPSS Inc., Chicago, IL, USA) was used for statistical evaluation. For all of the four groups; namely the nasal cavity, maxillary sinus, ethmoid sinus and control groups; the difference for eNOS PI between groups was analyzed by Kruskal-Wallis Variance Analysis. When a statistically significant result was found, pairwise comparisons were done by "Mann Whitney U Test" with Bonferroni correction to detect the value of group which had caused difference.

The confounding factors (Covariates age, gender, polyp duration, smoking and Brinkmann Index, rate of PMNC, rate of MNC, eNOS-PMNC-PI, eNOS-MNC-PI, eNOS-All Cells-PI) affecting eNOS-PI in layers of mucosa (Epithelial, subepithelial and deep) were analyzed by Linear Regression Analysis (Backward) (Step by Step Analysis) in the polyp groups (Group 1-3) totally. p value < 0.05 was considered as statistically significant.

All steps of the study were planned and conducted with approval of Kirikkale University Faculty of Medicine local Ethic Committee and according to the principles outlined in the Declaration of Helsinki[19]. This study was supported by "Kirikkale University Scientific Research Projects Unit Funds".

Results

eNOS Positivity Index values in Epithelial (apical, basal), subepithelial (perivascular, gland and endothelial) and deep (perivascular, gland and endothelial) layers of Ethmoid Sinus, Maxillary Sinus, Nasal Cavity and Control Groups; and rate of MNCs and eNOS-PI, rate of PMNCs and eNOS-PI and all cells eNOS-PI were demonstrated as median on Table I and Figures 1 to 3. As seen in Table I, eNOS Positivity Index median values were higher in epithelial-apical layer. While reaching from epithelial layer to the subepithelial and deep layers, median values of eNOS Positivities were decreased.

eNOS Positivity Index was intensive in apical part of the epithelium. Positivity was decreased from subepithelial to deep layers of lamina propria. In Groups 1-3 (Sinonasal Polyp Groups), as less cell density was present due to stromal edema, eNOS positivity in subepithelial and deep layers of lamina propria was observed less than the control group (see on Figure 1). eNOS expression in mononuclear cells was prominent in sinonasal polyps. In polimorphonuclear cells, almost no expression was observed in light microscopic exami-

Table I. eNOS Positivity Index Levels in Ethmoid Sinus, Maxillary Sinus, Nasal Cavity and Control Groups

	Groups				
eNOS Positivity Index	Ethmoid sinus (n=14) Median	Maxillary sinus (n=10) Median	Nasal cavity (n=10) Median	Control (n=9) Median	P*
eNOS_EP_apical	3.0	3.0	3.0	3.0	0.353
eNOS_EP_basal	1.0	1.0	0.0	1.0	0.164
eNOS_SE_pv	2.0	2.0	2.0	2.0	0.870
eNOS_SE_gland	1.5	1.0	1.0	2.0	0.125
eNOS_SE_endothelial	1.0	0.5	1.0	2.0	0.253
eNOS_D_pv	1.0	1.0	1.0	2.0	0.256
eNOS_D_gland	1.0	1.0	0.5	2.0	0.058
eNOS_D_endothelial	1.0	0.0	0.0	1.0	0.267
Inflammatory cells					
Rate of PMNCs	30.0	40.0	40.0	5.0	0.006
eNOS-PMNC	0.0	0.0	0.0	0.0	0.348
Rate of MNCs	70.0	60.0	60.0	90.0	0.459
eNOS-MNC	1.0	1.0	1.0	0.0	0.691
eNOS-All_Cells	2.0	2.0	2.0	1.0	0.046

*p value shows the results of Kruskal Wallis Variance Analysis

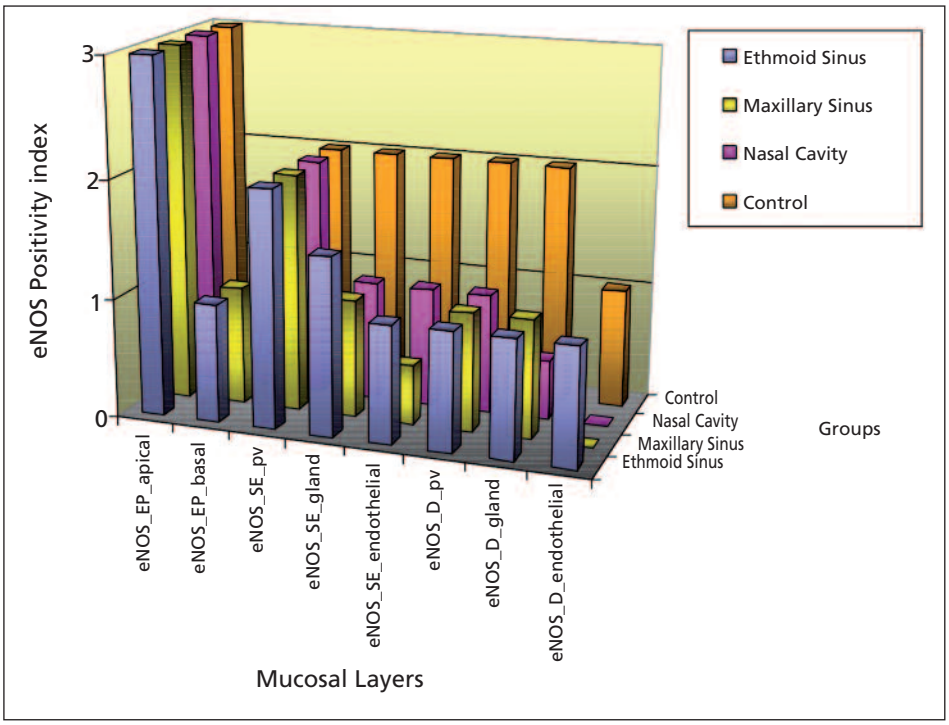

Figure 1. eNOS Positivity Index levels in mucosa of Groups 1-4.

nation (see on Figure 2). eNOS Positivity Index levels were examined in inflammatory cells of the mucosa. eNOS Positivity was higher in all cells group which was consisted of mainly mononuclear cells and fibroblasts, than only mononuclear cells. In polimorphonuclear cells, no eNOS positivity was detected (see on Figure 3).

The difference for eNOS PI values at eight layers of mucosa between all four groups was analyzed by Kruskal-Wallis Variance Analysis. No statistically significant difference was found between groups (Table I). The difference between rate of the inflammatory cells (rate of PMNCs and MNCs); and eNOS Positivity Index of PM-

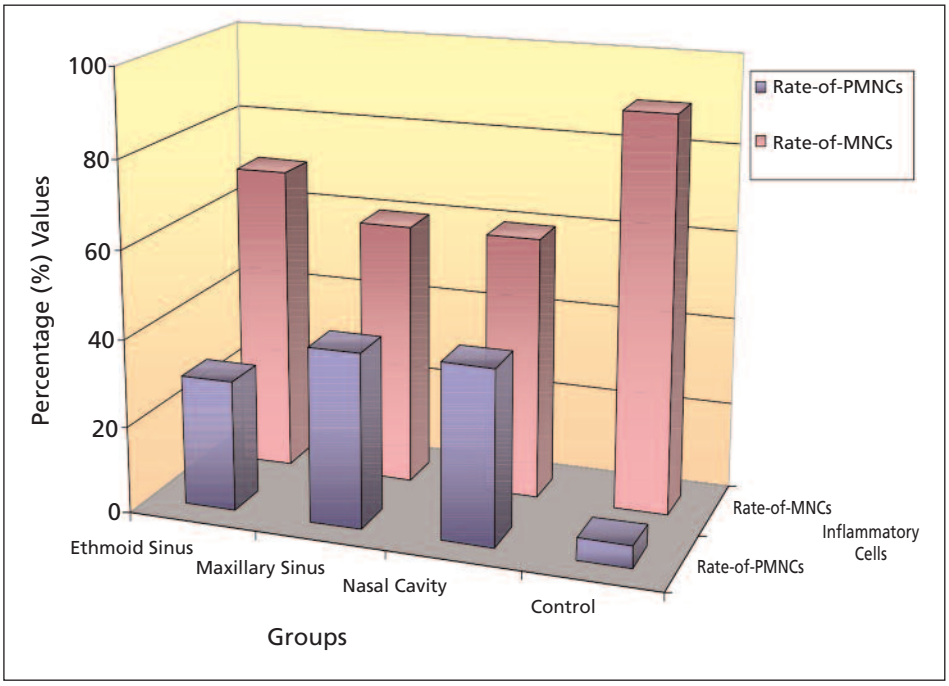

Figure 2. Distribution of Inflammatory Cells in Mucosa.

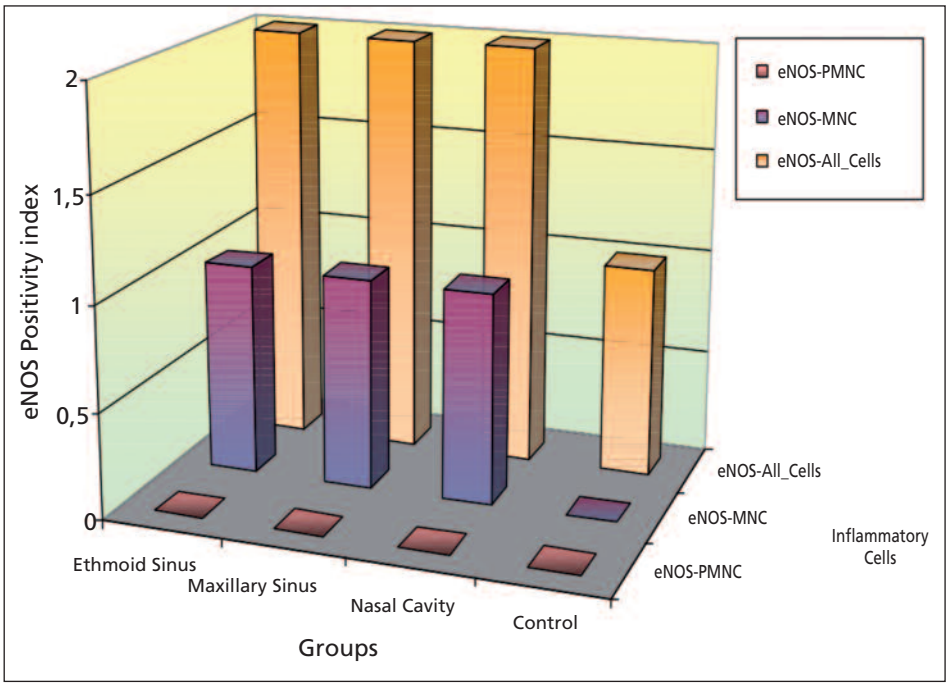

Figure 3. eNOS Positivity Index Levels in Inflammatory Cells.

NC, MNC and all cells was analyzed by Kruskal Wallis Variance Analysis; the statistically significant difference was found for PMNC-% (p = 0.006) and eNOS-All_Cells (p = 0.046) between Groups 1-4 (Table I).

To find the value which caused the difference, pairwise comparisons were performed by Mann Whitney U test with Bonferroni Correction. Rate of PMNC values of ethmoid sinus (Median 30.0) (p = 0.002); maxillary sinus (Median 40.0) (p = 0.008); and nasal cavity (Median = 40.0) (p = 0.003) were significantly higher than that of the control group (Median 5.0). eNOS-PI of all cells of nasal cavity (Median: 2.0) (p = 0.007) was significantly higher than that of the control group (Median 1.0) (Table II).

In the study group (Groups 1-3), the confounding factors (Covariates age, gender, polyp duration, smoking and Brinkmann Index, rate of PMNCs, rate of MNCs, eNOS-PMNC, eNOS-MNC, eNOS-All Cells) affecting eNOS-PI in layers of mucosa (Epithelial, subepithelial and deep) were analyzed by Linear Regression Analysis (Backward) (Step by Step Analysis) (Table III):

- **For eNOS_EP_apical:**

1. As polyp duration (years) increased, eNOS_EP_apical significantly decreased (p = 0.001, Beta = –0.652)

- **For eNOS_EP_basal:**

1. As rate of MNC increased, eNOS_EP_basal also increased (p = 0.003, Beta = 0.587)

- **For eNOS_SE_gland:**

1. In males, eNOS_SE_gland PI values decreased (p = 0.032, Beta: –0.385)
2. As eNOS-MNC PI increased, eNOS_SE_gland PI values were also, increased (p = 0.020, Beta = 0.469)

Table II. Pairwise comparisons by Mann Whitney U test with Bonferroni correction.

	Ethmoid Sinus-Maxillary Sinus		Ethmoid Sinus-Nasal Cavity		Ethmoid Sinus-Control		Maxillary Sinus-Nasal Cavity		Maxillary Sinus-Control		Nasal Cavity-Control	
	z	p	z	p	z	p	z	p	z	p	z	p
Rate of PMNCs	-0.560	0.576	0.000	1.000	-3.139	0.002	-0.228	0.820	-2.640	0.008	-2.962	0.003
eNOS-All_Cells	0.000	1.000	-0.638	0.524	-2.266	0.023	-0.484	0.629	-1.894	0.058	-2.709	0.007

Table III. Linear Regression (Backward) Analysis of factors affecting PDGF-PI levels at eight layers of mucosa in polyp groups (Group 1-3) (n=34)*.

Linear Regression (Backward) Analysis Results Covariates affecting eNOS Positivity Index Levels								
eNOS_EP_apical			**eNOS_EP_basal**			**eNOS_SE_pv**	**eNOS_SE_gland**		
Covariates	**Beta**	**p**	**Covariates**	**Beta**	**p**	**Covariates**	**Covariates**	**Beta**	**p**
Age	0.292	0.095	Localization of Polyp (Code 1: Ethmoid Sinus Code 2: Maxillary Sinus Code 3: Nasal Cavity)	–0.303	0.089	**No confounding factors was found**	Gender	–0.385	0.032
Polyp duration (years)	-0.652	0.001	Rate of MNCs	0.587	0.003		eNOS-MNC Positivity Index	0.469	0.020
			eNOS-MNC Positivity Index	0.358	0.056		eNOS-All Cells-Positivity Index	–0.473	0.019
eNOS_SE_endothelial			**eNOS_D_pv**			**eNOS_D_gland**	**eNOS_D_endothelial**		
eNOS-MNC Positivity Index	0.568	0.012	**No confounding factors was found**			Rate of MNCs 0.393 0.058	**No confounding factors was found**		
eNOS-All Cells-Positivity Index	-0.367	0.092							

*Covariates: Age, gender, polyp duration, Localization of Polyp, smoking and Brinkmann Index, rate of PMNCs, rate of MNCs, eNOS-PMNC-PI, eNOS-MNC-PI, eNOS-All Cells-PI

3. As eNOS-All Cells-PI increased, eNOS_SE_gland PI values decreased (p = 0.019, Beta = –0.473)

- **For eNOS_SE_endothelial:**

1. As eNOS-All Cells-PI increased, eNOS_SE_endothelial values decreased (p = 0.012, Beta = 0.568)

Histopathologic Findings

On light microscopic examination, it was observed that pseudostratified ciliated epithelium was present in majority of polyps; and a very small part of polyps was also lined with metaplastic epithelium. In eNOS stained sections, strong positivity was specifically detected in the apical portion of the surface epithelium. In subepithelial areas both the mucosal gland epithelium and endothelium showed diffuse immunostaining with eNOS. While mononuclear cells and fibroblast showed prominent eNOS expressions, almost no expression could be detected in polimorphonuclear leukocytes. In edematous areas of polyps, expression of all markers was found to be less than those in other areas. This was concluded to be due to the small number of cells in these edematous areas (Figures 5 and 6).

Discussion

Endothelial NOS (eNOS), also known as nitric oxide synthase 3 (NOS3), generates NO in blood vessels and is involved with regulating vascular function. A constitutive Ca^{2+} dependent NOS provides a basal release of NO. eNOS is associated with plasma membranes surrounding cells and the membranes of Golgi bodies within cells. eNOS localisation to endothelial membranes is mediated by cotranslational N-terminal myristoylation and post-translational palmitoylation[20].

Decrease in nasal NO production in patients with chronic rhinosinusitis was described by Lindberg, et al[21]. In Arnal et al's study[22], nasal NO concentrations in nonallergic patients with

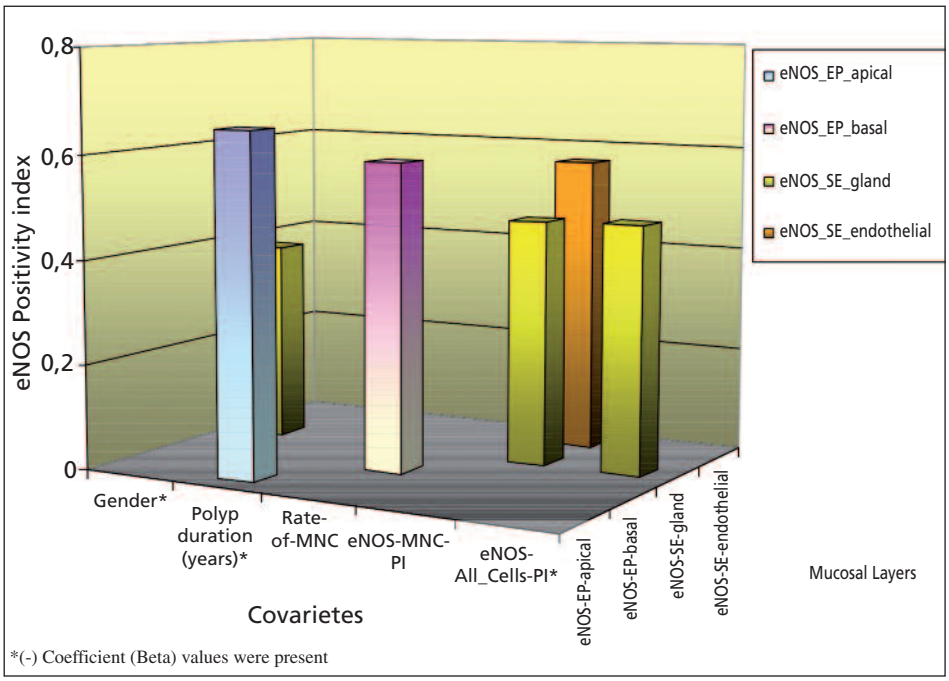

Figure 4. Coefficient (Beta) values and Covariates as significantly detected confounding factors on eNOS Positivity Index detected by Linear Regression Analysis (Backward).

nasal polyposis were significantly decreased compared with controls; and a correlation was found between the degree of obstruction of the paranasal sinuses and nasal NO measurements. Thébaud, et al[23] reported that NO, measured in the exhaled breath, originates from the paranasal sinuses. Exhaled NO is increased in nasal allergy and decreased in cystic fibrosis, nasal polyposis and chronic sinusitis[23]. Kharitonov, et al[24] reported that in subjects with acute upper respiratory tract infection, amount of NO in exhaled air is significantly increased. These differences on NO levels may be related to sinusal ostia patency: NO levels increased in acute respiratory tract infection. When the ostium is partially closed, the NO flow from the sinus decreases and, consequently NO concentration, measured in the nose exhaled air, decreases. The decrease in NO levels

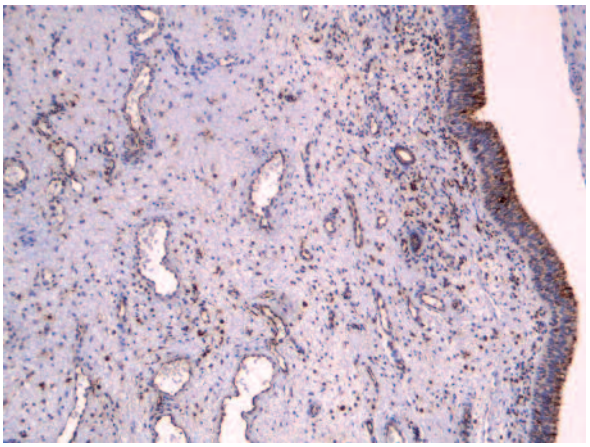

Figure 5. Immunohistochemical staining for eNOS in polyp sample displaying expression at epithelial apical layer mainly. In subepithelial layer, positive staining was observed in MNCs, vascular endothelial cells and perivascular region. Expression of eNOS was observed as intensive just below the basal membrane; and positivitiy was decreased from subepithelial to deep layers of lamina propria due to stromal edema and less cell density (x100).

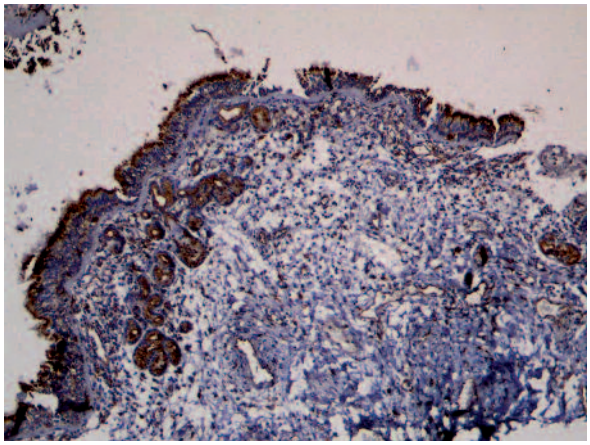

Figure 6. Immunohistochemical staining for eNOS in polyp sample showed that expression for eNOS was seen at apical part of epithelial layer; MNCs, gland epithelium and endothelial cells of subepithelial layer of mucosa (x100).

in chronic rhinosinusitis and nasal polyposis may be explained like this.

In the present study, we investigated the role of eNOS in the pathogenesis of nasal polyps. In the study and control groups, surgical specimens were examined with immunohistochemical staining technique using monoclonal antibodies against eNOS. No statistically significant difference for eNOS PI values was found at eight layers of mucosa between all four groups. As seen in Figure 1, in Groups 1-3 (Sinonasal polyp groups), eNOS PI was higher at apical layer of epithelium; and perivascular and glandular parts of subepithelial layer.

In the our study, eNOS expression was observed as intensive just below the basal membrane; and positivitiy was decreased from subepithelial to deep layers of lamina propria due to stromal edema and less cell density. In Groups 1-3 (Sinonasal Polyp Groups), as less cell density and/or less expression of eNOS caused lower eNOS positivity in subepithelial and deep layers of lamina propria compared to the control group (see on Figure 1). Decreased eNOS levels in nasal polyp may be related to obstruction of sinus ostia patency[22-24].

Similar to our results, Chen and Xiang[25] reported that in the nasal polyp group, eNOS located in gland cells, epithelial and vascular endothelial cells, the staining of which was a little stronger than those of controls. eNOS may play a role in glandular secretion and vascular dilatation. The increase of eNOS might respond to the enhanced nasal secretion and edema of nasal mucosa. In another study[26], expression of nitric oxide synthase (NOS) was significantly higher in nasal polyps than in nasal mucosa. eNOS reaction products were located in nasal polyps and nasal mucosa. There was no quantitative difference of eNOS between in nasal polyps and nasal mucosa. In the present study, the similar results were achieved to Sun, et al's study[26]. In our study, no significant difference for eNOS levels was present between the polyp and control group. Furukawa et al[1] demonstrated strong immunostaining for Type III NOS was localized to vascular endothelium, surface epithelium, and submucosal glands in inferior nasal turbinates of all subjects.Their findings demonstrate the cellular expression of NOS in the human nasal mucosa and suggest a possible role for Types II and III NO synthase in the regulation of blood flow, nasal secretion, and ciliary movement in health and disease.

By Linear Regression Analysis (Backward), the most affecting factors of eNOS levels were analyzed: (1) In older polyps, eNOS positivity index values at epithelial apical layer decreased. (2) In patients with increased rate of MNCs, eNOS positivity index values at epithelial basal layer increased; and as eNOS-MNC positivity index increased, eNOS values also increased at glands of subepithelial layer. (3) In males, eNOS values decreased at glands of subepithelial layer. (4) As eNOS-all cells positivity index increased, glandular and endothelial eNOS values decreased.

In our study, when the polyp duration gets longer, eNOS values declined especially at the apical part of surface epithelium. eNOS has an impact on mucosa by increasing permeability of vascular endothelium and by increasing the secretion of glands. In patients with long-term polyps, less edematous and more fibrotic polyps may grow. In male polyp group, glandular eNOS values decreased at SE layers. Men may exposure to different irritants at work and in traffic; and these conditions may play a role in difference between males and females in our study.

NO modulates nasal immunology, influences macrophage activity, and has antiviral and bacteriostatic properties. Nitric oxide in nerve fibers, seromucous glands, and endothelial cells of capillaries and arterial vessels suggest that NO takes part in the regulation of physiologic processes of the human nasal mucosa[3]. eNOS and the NO molecule are involved in cell cycle regulation, in the apoptotic processes and cell proliferation, as well as in the angiogenesis and vasculogenesis. eNOS is expressed in the tissues of the upper airways in both chronic inflammation and carcinomatous processes[27].

In our study, rate of PMNC values of study group; and all cells eNOS positivity index in nasal cavity were higher than the control group. We observed that eNOS was not expressed in PMNC (mainly neutrophils); and was expressed in MNCs and all cells group. In all cells group, higher eNOS expressions compared to MNCs were due to fibroblasts; and higher eNOS expressions in fibroblasts (Figure 3). Increased MNCs and MNC positivity index for eNOS are seen with increased eNOS expression at epithelial-basal level. As eNOS-All cells-PI increased, mainly fibroblasts constitute this group, glandular and endothelial eNOS values decreased in deep layer of the mucosa.

It is well-known that neutrophils are growing more in acute inflammatory process. MNCs and fibroblasts are cells involved in chronic inflammatory process. And, as the nasal polyp growing is a chronic inflammatory process, chronic inflammatory cells increased in nasal polyps; and eNOS expressed from cells of chronic inflammatory process was more dominantly detected by immunohistochemical analysis. In our study, one group of the cells of chronic inflammatory process is MNCs, going on with increased eNOS expression at epithel-basal layer; the other cell group of chronic inflammation is fibroblasts, going with decreased eNOS expression at glands and endothelial cells of the mucosa. As a hypothetical, polypoid process may be initiated by mononuclear cells and increased eNOS levels expressed from MNCs with the result of edematous polyps; in the further period of chronic inflammation and polypoid formation, fibroblasts increased; eNOS expression from fibroblasts decreased and at the end, less edematous and more fibrotic polyps develop.

In the literature, reports about effects of age and smoking on nasal NO were present: Nasal NO concentrations are influenced by age, physical exercise, smoking and certain drugs. Nasal NO is conveniently measured in all ages and can be used for screening of disease or monitoring the effects of treatment. Pathological conditions, as in allergic rhinitis, sinusitis, nasal polyps, cystic fibrosis and primary ciliary dyskinesia, result in altered nasal NO concentrations. The clinical relevance for measurement of nasal NO in different conditions, however, remains to be established[28]. In our study, Linear Regression Analyses revealed no additional confounding effects of smoking on eNOS values.

Cannady et al[29] investigated the level of expression of the endothelial nitric oxide synthase (eNOS)/soluble guanylate cyclase (sGC) system in nasal polyps and control nasal mucosae. The study was performed in polyps from 15 patients and nasal mucosae from 11 subjects operated on the nasal septum (control group). The expression of endothelial nitric oxide synthase (eNOS) and soluble guanylate cyclase (sGC) was determined in nasal mucosae. They found that eNOS protein was overexpressed in the nasal polyps with respect to control nasal mucosae. Immunohistochemistry also demonstrated that the vascular endothelium of nasal polyps contained higher amounts of eNOS protein than control nasal mucosa. Moreover, the beta subunit of sGC was also overexpressed in the nasal polyps, which was associated with an increased content of cyclic GMP in the nasal polyps with respect to nasal control mucosae. They concluded that, in human nasal polyposis, there is an overexpression of the eNOS/sGC system. Further studies are needed to assess whether this overexpression is involved in the genesis of nasal polyposis.

NO was colocalized in parasympathetic nerves and plays a role in the neurotransmission and neuromodulation of the vascular tone and glandular secretion. Arteries showed a distinctly developed nitric innervation and endothelial accumulation. The NO production in axons of the adventitia and in the endothelium of arteries demonstrated that these vessels are influenced by a dual NO system. Mainly NO could act on these structures with vasodilatory effects[3,5,14].

eNOS play role in the regulation of blood flow, nasal secretion, and ciliary movement in health and disease[1]. Moreover, the presence of nitric oxide (NO) in high concentrations has been described in the nasal mucosa of patients with untreated allergic rhinitis. NO stimulates collagen expression in human nasal polyp-derived fibroblasts. This stimulation appeared to favor the up-regulation of collagen type III, leading to a shift in the ratio of collagen type I to type III production[30].

In our study, the apical parts of epithelial eNOS values were higher, but eNOS values at SE and deep parts were not very high. Simiar to our results, Cannady, et al[29] reported that NO metabolite levels (nitrite and nitrate) in nasal lavage fluid from patients with polyps were less than those in control subjects, but activation of signal transduction and inducer of transcription 1, which regulates inducible NOS gene expression and protein expression, was present at higher levels in polyp than in healthy control tissue. In this case, signal transduction and inducer of transduction issues needs to be explored in more detail.

In our study, eNOS PI was higher at apical layer of epithelium; and perivascular and glandular parts of subepithelial layer. eNOS plays role in vascular dilatation, increase in vascular permeability; increase in nasal secretion by glandular secretion; and edema in SE and deep layers of the mucosa by affecting glands. Irritant agents in the breathing air and environment may cause increase in eNOS values at apical part of epithelium and promotes polyp formation by vasodilata-

tion and increased glandular secretion due to increased nitric oxide values. In patients with older polyps, eNOS values decreased, causing more fibrotic polyps.

NO, by activating the enzyme Cox-II, increases the inflammatory prostaglandin synthesis. NOS inhibitors prevent edema and vascular permeability increase in inflammation in a dose-dependent manner[31]. NO synthase (NOS) inhibitors, and selective endothelial cell NOS inhibitor L-N (1-iminoethyl)-ornithine[32] systemically ameliorated the severity of inflammation throughout the reaction. NOS inhibitors have anti-inflammatory effects depending on their route of administration. In future studies, the effects of NOS inhibitors on polyp pathogenesis; and also in patients applied medical or surgical treatment for sinonasal polyps should be investigated

Conflict of Interest

The authors declare that there is no conflict of interest.

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