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# Expression of Vascular Endothelial Growth Factor in Nasal Polyp and Chronic Rhinosinusitis

Alireza Azizzadeh Delshad, Mohammadreza Jalali Nadoushan, Ali Davati, Aida Rostami

Dept. of Anatomical Sciences and Pathology, Faculty of Medicine, Shahed University, Tehran, Iran

## KEY WORDS

VEGF  
Nasal polyp  
Chronic rhinosinusitis

## ABSTRACT

**Background:** Nasal inflammatory disorders such as chronic rhinosinusitis and nasal polyp are among the most prevalent complications with high socioeconomic costs. Vascular Endothelial Growth Factor (VEGF) plays a key role in angiogenesis and cell proliferation. In the present study the effect of VEGF on the development and prognosis of chronic rhinosinusitis and nasal polyp was investigated.

**Methods:** This cross sectional study was performed on the nasal histological specimens of two groups of patients suffering from nasal polyp or chronic rhinosinusitis, and the expression of VEGF in the two groups was compared immunohistochemically. Based on the percentage of VEGF-positive cells the specimens were classified into four scores. Furthermore, the relations between the VEGF expression and some demographic characteristics were evaluated.

**Results:** The VEGF immunohistochemistry findings indicated a significantly higher expression of VEGF in nasal polyp group compared to chronic rhinosinusitis without nasal polyp group. In terms of VEGF-expression scoring, in both groups most of the specimens were classified as score-2, namely indicating 10-50% of VEGF-positive epithelial cells. In both groups no significant relation between VEGF expression and age or sex of the patients could be seen.

**Conclusion:** Local modulation of VEGF expression might be taken as a putative therapeutic strategy in management of sinunasal inflammatory disorders, especially nasal polyps.

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**Corresponding Information:** Dr. Alireza Azizzadeh Delshad, Department of Anatomical Sciences and Pathology, Faculty of Medicine, Shahed University, Opposite Holy Shrine of Imam Khomeini, Khalij Fars Expressway, P.O. Box: 18155/159, Tehran, Iran, delshada@yahoo.com, Tel.: 021-88964792

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## Introduction

The coexistence of rhinitis and sinusitis is called rhinosinusitis defined as inflammation of the nose and paranasal sinuses with two or more of the following symptoms: nasal congestion or blockade, anterior or posterior nasal discharge, facial pain or pressure, reduction or loss of smell, and complementary endoscopic signs and CT changes. The ostiomeatal complex plays a fun-

damental role in the pathogenesis of rhinosinusitis (1). If rhinosinusitis persists for more than 12 weeks, it would be classified as chronic rhinosinusitis (CRS) (2), which can be the consequence of epithelial damage and activation of immunity (3). Nasal polyps (NPs) are grape-like structures, consisting of loose connective tissue, oedema, inflammatory cells, glands and capillaries, and are usually covered with respiratory pseudostratified epithelium and goblet cells, and include many

inflammatory cells such as neutrophils, mast cells, plasma cells, lymphocytes, monocytes, fibroblasts, and specially eosinophils as the most common cells (1). NP can be associated with allergy, asthma, aspirin sensitivity, cystic fibrosis and infection (4)

Since it is impossible to differentiate between NP and CRS, they are often taken together as one disease entity (5), and NP is considered a subgroup of CRS. It is unknown whether chronic rhinosinusitis, gives rise to polyp growth, or these two conditions happen independently. However, for clinical purposes, these pathological nasal conditions can be differentiated as acute rhinosinusitis (ARS), chronic rhinosinusitis with Nasal Polyp (CRSwNP) and chronic rhinosinusitis without Nasal Polyp (CRSnNP).

CRS can be accompanied with or without nasal polyps (NP), which although similar in their symptoms, follow different inflammatory patterns (2), and are different at molecular and cellular level. Due to the insufficient phenotypic differentiation of different types of CRS, it is necessary to differentiate between them by cellular and molecular markers (6), which can be useful to better predict the patient prognosis and to develop new effective therapies.

CRSwNP is characterized by neutrophil infiltration, increased fibrosis, and collagen deposition in the stroma and a slightly thickened basement membrane; whereas CRSnNP is defined by extensive leukocyte infiltration with overt presence of pseudocysts, albumin accumulation and edema, decreased collagen in stroma, thickened basement membrane and significant epithelial alterations (7). The two types of CRS with and without NP not only have different inflammatory patterns, but also it seems that they show different responses to treatment, namely CRSwNP tend to react better to nasal and systemic corticosteroids (8), whereas in the case of CRSnNP, long-term therapy with macrolides can be more effective (9).

Vascular endothelial growth factor (VEGF),

a ~45 kDa dimeric heparin-binding glycoprotein with pro-oedematous and angiogenic properties, plays a key role in angiogenesis and vessel remodeling. VEGF specifically binds to high-affinity receptors with tyrosine kinase activity. VEGF induces endothelial cell proliferation and increases vascular permeability and is involved in wound healing, tumour growth, and chronic inflammation (10). VEGF through inducing vascular hyperpermeability, edema and angiogenesis (11), can play a key role in pathogenesis of inflammatory disorders such as nasal polyp (10).

In the present study, by comparison of VEGF expression in NP and CRSnNP, its worth as a useful biomarker in diagnosing different types of inflammatory nasal disorders was investigated.

## Materials and Methods

This cross sectional study was performed according to the ethical guidelines of Shahed University Research Ethics Committee, on pathological specimens of 103 patients having CRSwNP and 30 patients suffering from CRSnNP.

All patients underwent endoscopic endonasal surgery, and a sample of excised tissue was prepared for histopathological investigation. The samples with a diagnosis of malignancy as well as congenital angiogenic pathological conditions were excluded from the study. The tissue samples were fixed in formaldehyde, embedded with paraffin and 3  $\mu$ m sections were obtained. From all specimens one section was stained with hematoxyline & eosine to confirm the pathological characteristics, and another section was prepared for immunohistochemical investigation of VEGF.

### VEGF immunohistochemistry

Following deparaffinization and rehydration, the sections were exposed to 3% peroxide hydrogen in methanol for 10 min. To retrieve the antigen, the sections were put in buffer citrate (pH:6)

and placed in a microwave autoclave adjusted to 126 °C and 15 atmospheric compression for 1 min. Then the sections were washed by TBS (pH: 7.2-7.6), and were exposed to peroxidase and protein blocking solutions, each for 5 min. At the next stage the samples were covered with anti-VEGF-antibody (Novocastra/UK) for 1 h at room temperature, and then placed in post-primary blocking solution for 30 min. Then the sections were incubated with polymer solution of secondary antibody for 30 min and washed with TBS. Finally the samples were covered for 5 min with diaminobenzidine (DAB) as chromogen and counterstained with hematoxyline for about 30 sec.

At the end, the sections were mounted and under a light microscope the percentage of VEGF-positive epithelial cells were calculated and scored by a semiquantitative scale: No positive cells was considered as score-0, and less than 10%, 11-50%, and more than 50% positive cells were regarded as scores 1, 2 and 3, respectively. Furthermore based on the percentage of the VEGF-positive cells, with a cutoff point of 10%, the specimens were divided into negative (less than 10% positive cells) and positive states (more than 10% positive cells). The demographic data of the patients were also gathered and analyzed in both groups.

### Statistical analysis

The obtained data were analyzed by statistical tests Chi-square, Mann-Whitney, Sperman, one-

way ANOVA and t-test, and  $P < 0.05$  was considered significant.

## Results

This study was performed on 133 patients, 103 with NP and 30 cases suffering from CRSnNP. In both groups the percentage of male and female patients was almost the same (Table 1).

In the NP-group, the youngest and oldest patients were 19 and 88 yr old, whereas in the CRSnNP-group these values were 18 and 60 yr. Table 2 summarizes the mean and standard deviation of the age of patients in the two groups and the total values.

The four immunohistochemically VEGF-positivity-scores, did not show equal prevalences. In both NP and CRSnNP groups, most of the cases showed the score-2 (87.4% of NP-group and 66.7% of CRSnNP-group), whereas no score-0 could be identified in each of the groups. 5.8% of NP-group and 33.3% of CRSnNP-group showed score-1. 6.8% of NP-group indicated score-3 whereas none of the CRSnNP-group showed score-3 (Fig. 1). Chi-square test indicated significant difference between two experimental groups, in terms of VEGF-positivity-scoring ( $P < 0.001$ ).

Based on the VEGF-positivity, the negative (less than 10% positive cells) and positive (more than 10% positive cells) states were calculated in both groups. In NP-group 94.2% of cases were estimated positive and only 5.8% were negative, whereas in the CRSnNP-group 66.7% were

**Table 1**

The prevalence and percentage of genders in both Nasal Polyp (NP) and Chronic Rhinosinusitis without Nasal Polyp (CRSnNP) groups.

Group		Male	Female	Total
NP	Number	64	39	103
	Percentage	62.1	37.9	100
CRSnNP	Number	19	11	30
	Percentage	63.3	36.7	100
Total	Number	83	50	133
	Percentage	62.4	37.6	100

**Table 2**

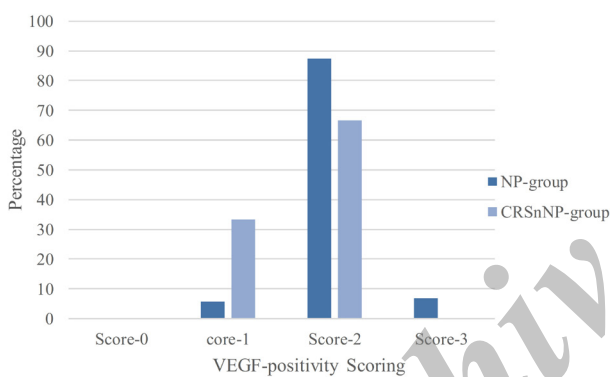
The mean and standard deviation of age of both Nasal Polyp (NP) and Chronic Rhinosinusitis without Nasal Polyp (CRSnNP) groups in different genders.

Group	Male	Female
NP	46.95 ± 16.08	38.74 ± 14.9
CRSnNP	35.16 ± 10.36	39.64 ± 13.94
Total	44.25 ± 15.72	38.94 ± 14.56

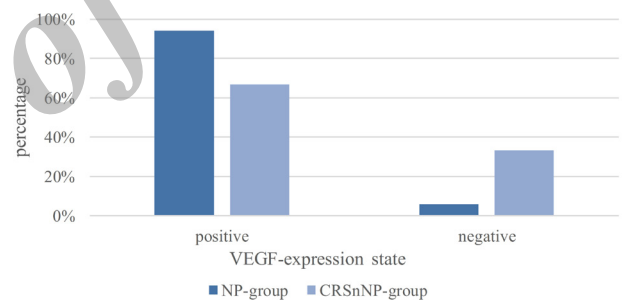
**Table 3**

Prevalence and percentage of VEGF scores in both Nasal Polyp (NP) and Chronic Rhinosinusitis without Nasal Polyp (CRSnNP) groups

Group	Score 0	Score 1	Score 2	Score 3	Sum	
NP	Number	0	6	90	7	103
	Percentage	0	37.5	81.8	100	77.4
CRSnNP	Number	0	10	20	0	30
	Percentage	0	62.5	18.2	0	22.6
Total	Number	0	16	110	7	133
	Percentage	0	100	100	100	100

**Fig. 1**

Percentage of different VEGF-positive scores, in both Nasal Polyp (NP) and Chronic Rhinosinusitis without Nasal Polyp (CRSnNP) groups

**Fig. 2**

Percentage of cases of both Nasal Polyp (NP) and Chronic Rhinosinusitis without Nasal Polyp (CRSnNP) groups, defined as positive or negative VEGF-expression states, with the cutoff point of 10% positive cell

positive and 33.3% were negative (Fig. 2). These data suggest that in the NP-group compared to CRSnNP-group, significantly more cases were positive. Statistical analysis by Mann-Whitney test indicated a significant difference in VEGF expression between NP and CRSnNP groups ( $P=0.005$ ).

Besides, the prevalence and percentage of all VEGF scores in both groups were calculated (Table 3), and analyzed by chi-square test, which indicated a significant relation between the two

experimental groups in terms of VEGF-expression scoring ( $P<0.001$ ).

Chi-square test indicated no significant differences between gender and VEGF scoring ( $P=0.422$ ), as well as between gender and VEGF-expression state ( $P=0.993$ ), which proposes that gender had no significant effect on VEGF expression. Furthermore, ANOVA test showed no significant relation between age and VEGF-positivity scoring ( $P=0.461$ ), t-test showed no significant relation between age and VEGF-expression state



( $P=0.679$ ), and finally the results of Spearman test also could not show any significant correlation between age and percentage of VEGF expression ( $P=0.3$ ). All of the following statistical results indicated that age too, had not any significant effect on VEGF expression.

## Discussion

The findings of our VEGF immunohistochemistry study on the histopathological samples of nasal mucosa indicated that in the NP specimens the expression of VEGF was significantly higher than in the chronic rhinosinusitis without NP samples. In terms of VEGF-expression scoring, in both groups most of the specimens were classified as score-2, namely indicating 10-50% of VEGF-positive epithelial cells. Investigating the demographic data indicated no significant relation between VEGF expression and age or sex of the patients in both groups.

In a study performed on 100 patients with NP, the age range of patients was from 14 to 70 yr, and 68% of patients were 20-50 yr old, and no significant relation existed between sex of the patients and the occurrence of NP (12). In another study on forty-four patients suffering from chronic rhinosinusitis and concomitant NP and asthma, a significant relation between NP and older age was characterized ( $P<0.01$ ) (13).

The nasal epithelium acts as a physical and immune barrier, and in the development of CRS in addition to external risk factors, the epithelial response is also important. Inflammatory stress would induce injury of nasal epithelium, followed by a rapid remodeling response including epithelial hyperplasia, goblet-cell metaplasia, loss of cilia, fibrosis, and basement membrane thickening (14).

In patients with CRSwNP, nasal symptoms such as impaired smell and nasal discharge are more common, whereas patients suffering from CRSnNP more often complain of facial pain. However these two clinical conditions have

many overlapping symptoms (15) which make their distinction difficult. In spite of many studies performed on the nasal inflammatory complications such as CRS or NP, the pathogenesis of these ailments has not been clarified exactly. The interaction of many genes and environmental factors may play key roles in the pathogenesis of these disorders. Genetic and molecular pathways involved in pathogenesis and prognosis of CRS, are going to be better understood. This understanding can be helpful in establishment and development of more effective therapeutic strategies for CRS treatment. The expression of involved genes in these inflammatory responses can be used as a marker to define the pathophysiological processes and/or the diagnostic and therapeutic procedures. One of these biomarkers is VEGF, which has been proved to be involved in proliferative and angiogenic processes. Lee and Kim examined the expression of members of VEGF genes and their receptors in cultured primary human nasal epithelial cells, and observed abundant expression of different isoforms of VEGF, which may function as additional pathways to promote growth of airway epithelial cells during inflammation (16). Hu et al. studied the expression of VEGF in a group of children suffering from CRSwNP and another group having CRSnNP, and indicated a significantly abundant expression of VEGF within NP tissue compared to CRS, and concluded that VEGF participates in formation of NP by inducing plasma extravasation and angiogenesis (17).

VEGF is one of the main angiogenic factors essential for embryonic vasculogenesis and/or angiogenesis (18), vascular permeability (19), endothelial cell proliferation (20), tumor angiogenesis (21), and is expressed in human tumors including lymphoma (22). VEGF activates endothelial cells through two main VEGF receptors: VEGFR-1 and VEGFR-2; VEGFR-1 appears to have both inhibiting and promoting roles in angiogenesis (23), whereas VEGFR-2 stimulates mitosis and motility of endothelial cells (24). In

addition to angiogenesis and edema, other basic mechanisms of tissue homeostasis, such as proliferation and apoptosis, might be involved in nasal polyp formation and regulated by VEGF. High levels of VEGF in polyps might activate VEGFRs and downstream pathways, resulting in increased proliferation and decreased apoptotic cell death, thereby promoting polyp formation (25).

## Conclusion

Parallel to the routine treatments, local modulation of VEGF expression might be taken as a putative therapeutic strategy in management of sinonasal inflammatory disorders, especially nasal polyps.

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## Conflict of interest

None of the authors have any conflict of interest to disclose.

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