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VIMENTIN EXPRESSION IN NASAL MUCOSA OF PATIENTS WITH EXACERBATED CHRONIC RHINOSINUSITIS WITHOUT NASAL POLYPS

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Abstract. Objective. *The aim of the study was to evaluate vimentin expression in inflamed nasal mucosa of patients with chronic rhinosinusitis without nasal polyps (CRSsNP) and serum levels of matrix metalloproteinase-9 (MMP-9).* ***Material and Methods.*** *We measured concentrations of MMP-9 in blood serum of twenty patients with CRSsNP using ELISA and compared them with the control group composed of twenty healthy subjects. Vimentin expression in nasal mucosa was studied by an immunohistochemical method.* ***Results.*** *Blood serum levels of MMP-9 were found to be elevated in patients with CRSsNP. The disease was also associated with the upregulation of vimentin expression both in the lamina propria and nasal epithelial layer.* ***Conclusion.*** *CRSsNP is accompanied by a higher number of vimentin-expressing cells in the nasal epithelium, which may indicate their epithelial-to-mesenchymal transition (EMT). We speculate that MMP-9 may contribute to the increased rate of EMT of nasal epithelial cells in CRSsNP.*

Key words: *chronic rhinosinusitis without nasal polyps, vimentin, matrix metalloproteinases, extracellular matrix remodeling*

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INTRODUCTION

Chronic rhinosinusitis (CRS) is characterized by prolonged inflammation of nasal mucosa and nasal sinuses [1-3]. The most common symptoms of the disease are excretions from the nose, nasal obstruction, olfactory decline, and facial pain. To verify the diagnosis, two out of four symptoms mentioned above should persist for at least 3 months [2]. The prevalence of CRS in developed countries is fairly high and may vary from 4.5% to 12% in line with different studies [4]. According to some authors, the prevalence of the disease reaches 16.55% in some regions (e.g., South America) [5]. It is believed that the average prevalence of CRS based on diagnostic criteria of the European Position Paper on Rhinosinusitis and Nasal Polyps (EP³OS) Europe is 10.9% [6]. The high incidence rate of the disease in population creates a high socioeconomic burden. According to various assessments, direct and indirect costs

associated with CRS exceed $20-22 billion USD per year in the United States [7, 8].

Classification of CRS is based on the presence of nasal polyps. According to this classification, two types of the disease are defined: chronic rhinosinusitis without nasal polyps (CRSsNP) and chronic rhinosinusitis with nasal polyps (CRSwNP) [9, 10]. According to reports the former is mainly characterized by Th1-mediated neutrophilic inflammation, while the latter is accompanied by Th2-associated eosinophilic inflammation [11]. However, it is believed nowadays that CRSsNP is associated with neither Th1-mediated, nor Th-2 mediated immune response [9].

Two types of CRS differ in tissue remodeling patterns. A. Kato has reported that the pivotal role in tissue remodeling in CRSsNP is played by transforming growth factor-β (TGF-β) and TGF-β-signaling pathways, while in CRSwNP it is primarily associated with the action of cytokines related to Th2-mediated immune response [10]. However, the process of extracellular matrix remodeling and epithelial-to-mesenchymal transition (EMT) involved in tissue remodeling in CRSsNP still remains poorly understood and their mechanisms should be elucidated.

The objective of our study was to evaluate vimentin expression in inflamed nasal mucosa of patients with CRSsNP and blood serum levels of matrix metalloproteinase-9 in patients with CRSsNP.

MATERIAL AND METHODS

1. Study design and groups of patients

Twenty patients with CRSsNP treated at Kharkiv Regional Clinical Hospital were recruited for our research. CRSsNP was verified according to recommendations of ‘‘EPOS 2012: European Position Paper on Rhinosinusitis and NPs 2012” guidelines [12]. We used twenty patients who underwent septal surgery as control subjects. Individuals with any clinical sign of sinonasal inflammation, other acute or chronic inflammatory diseases, immunodeficiency, endocrine diseases, chronic cardiovascular pathology, and tumors were excluded from the research.

Sinonasal tissue specimens were collected from nine patients with CRSsNP and seven control subjects to carry out the immunohistochemical study using antibodies to vimentin. Samples of blood were taken from both patients and control subjects.

2. Immunohistochemistry

Vimentin expression was immunohistochemically detected in specimens of sinonasal tissues collected during sinus surgery. Vimentin is a cytoskeleton protein whose expression is observed in mesenchymal cells only. The samples were fixed in 10% neutral formalin solution. Paraffin-embedded sinonasal tissues were used to obtain 4 μm-thick sections. In this study, mouse monoclonal antibodies produced by “Thermo Fischer Scientific” (UK) were used to analyze vimentin expression. Initially, microslides were incubated with the primary antibodies to vimentin. Then they were treated with anti-(mouse IgG)–horseradish peroxidase conjugate. We used 3,3′-diaminobenzidine (DAB) for visualization.

3. Van Gieson staining

Fixation of sinonasal tissue sections in 10% neutral formalin solution was followed by van Gieson’s picrofuchsin staining.

4. ELISA measurement of MMP-9 in blood serum

Levels of MMP-9 in blood serum of patients with CRSsNP were determined using commercially available ELISA kits by eBioscience (Vienna, Austria). The Awareness Technology Stat Fax 303 Plus Microstrip Reader (USA) was used to determine the optical density of solutions. MMP-9 concentrations were expressed in ngml.

5. Bioethics

Study design was developed and all manipulations were performed according to the ethical standards of the Committee of Ethics and Bioethics of Kharkiv National Medical University and the revised Declaration of Helsinki (2000). Informed consent was signed by all patients and control subjects who had been enrolled in the study.

6. Statistical analysis

Statistical analyses of data were carried out using “GraphPad Prism 5” software. The Mann-Whitney U test was used to compare two groups of independent variables. Data were presented as median and interquartile range. Differences were considered statistically significant if $p < 0.05$.

RESULTS

The study showed that the positive vimentin staining was primarily localized in the lamina propria of the nasal mucous membrane in healthy control subjects. Virtually no vimentin-expressing cells were observed in the epithelial layer (Fig. 1).

Analysis of samples showed that the number of vimentin-positive cells in the lamina propria was higher in patients with CRSsNP compared to the control specimens (Fig. 2a, 2b, 2c, 2d). However, the intensity of vimentin labeling was weak. In regions where the infiltrate was less pronounced, the intensity of vimentin labeling was markedly higher. The analysis

of nasal tissue sections stained by van Gieson confirmed our hypothesis about the secondary collagen destruction in areas with cellular infiltrate.

The epithelium of nasal tissues obtained from patients with CRSsNP contained some mesenchymal-like vimentin-expressing cells, which might indicate their EMT, taking into account the absence of vimentin expression in the normal epithelial cells (Fig. 2a, 2c).

In regions where the thick collagen fuchsin-positive layer between the lamina propria and epithelium was formed, EMT rate was reduced and a higher number of vimentin-expressing cells were observed in the lamina propria only. In addition, intense leukocyte infiltration was accompanied by the reduction of vimentin-positive cells in the lamina propria.

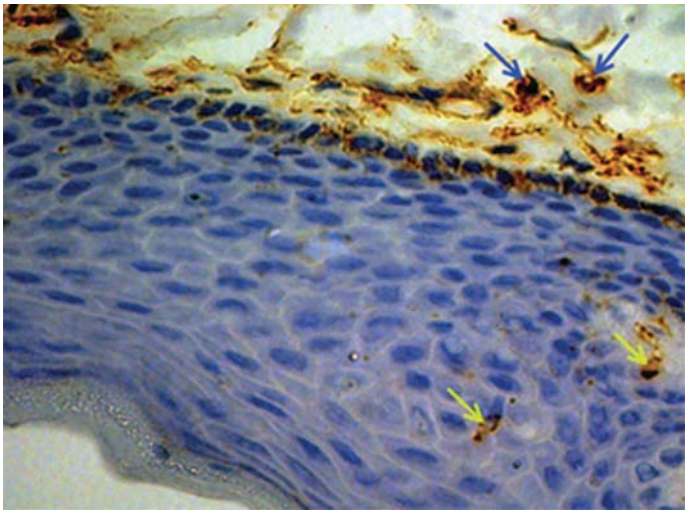

Fig. 1. Nasal mucosa of a healthy control subject. Epithelial layer is clearly visible. The number of vimentin-positive cells in epithelium is extremely low (marked with yellow arrows). Some weakly vimentin-positive fibrocytes are observed in the lamina propria (marked with blue arrows). Immunohistochemical reaction with antibodies to vimentin. x 400

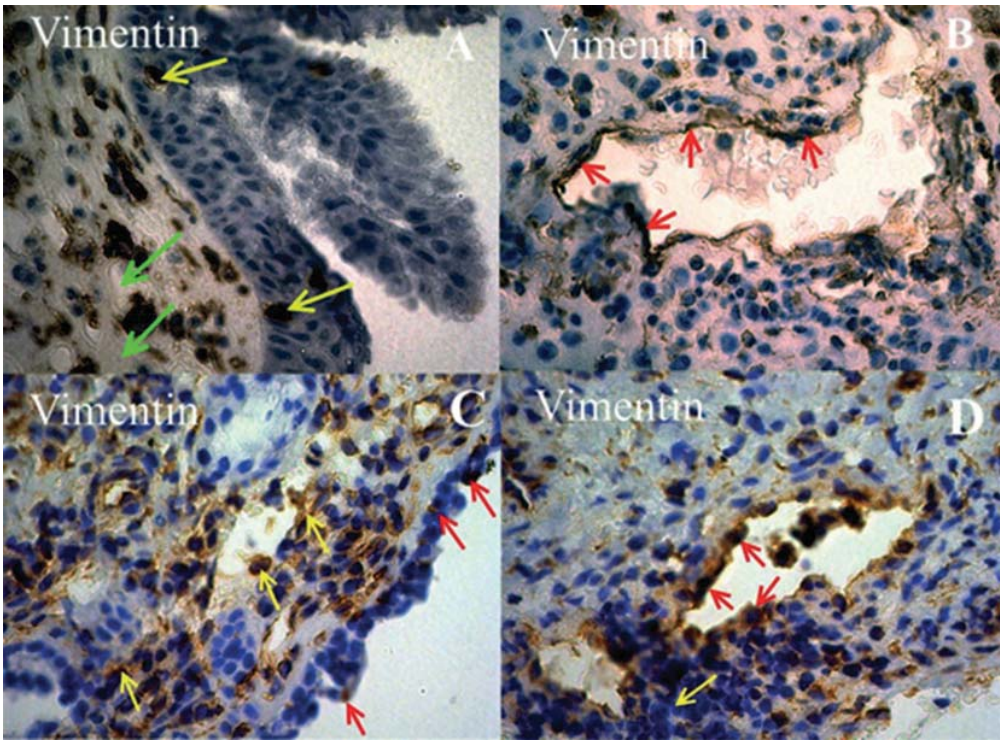

Fig. 2. A) Nasal tissue of a patient with CRSsNP without signs of neutrophilic inflammation with the pronounced sclerosis of the lamina propria (marked with green arrows). Some vimentin-positive cells are observed in the epithelial layer (marked with yellow arrows). Immunohistochemical staining with antibodies to vimentin. x 400. **B)** A small vein in a specimen of nasal tissue of a patient with CRSsNP. Numerous vimentin-positive pericytes form the outer layer of vascular walls (marked with red arrows). Immunohistochemical staining with antibodies to vimentin. x 400. **C)** A nasal tissue of a patient with CRSsNP. Vimentin-expressing cells are revealed in the atrophic epithelial layer (marked with red arrows). Intense vimentin labeling is observed in the lamina propria (marked with yellow arrows). Immunohistochemical staining with antibodies to vimentin. x 400. **D)** A nasal tissue fragment of a patient with CRSsNP. Vimentin-expressing pericytes are abundant in the outer layer of the vascular wall (marked with red arrows). Leucocyte infiltrates can be seen (marked with yellow arrows). Immunohistochemical staining with antibodies to vimentin. x 400

In this study, we found that the level of MMP-9 was 2.6-fold significantly higher ($p < 0.0001$) in blood serum of patients with CRSsNP compared to the control group (Table 1).

Table 1. Blood serum concentrations of matrix metalloproteinase – 9 in blood serum of patients with CRSsNP

Parameter	Healthy subjects (n = 20)	CRSsNP (n = 20)
Matrix metalloproteinase – 9 (MMP-9), ng/ml	2.73 [1.52; 4.75]	7.16 [6.50; 9.30] P < 0.0001

Note: p is a significance value compared to the control group

DISCUSSION

The difference between vimentin expression patterns in the epithelial layer in nasal mucous membranes of healthy individuals and patients with CRSsNP indicated that some nasal epithelial cells in CRSsNP acquired EMT-associated features, since vimentin is expressed only in mesenchymal cells and is considered to be a marker of EMT. It is conceivable that this EMT-like process in the nasal epithelium that accompanies the development of CRSsNP plays an important role in its pathogenesis.

Matrix metalloproteinases, including MMP-9, are proteolytic enzymes crucial for the degradation of extracellular matrix components [13, 14]. Our data confirm the important role of MMP-9 in tissue remodeling in CRSsNP. MMP-9-dependent proteolysis may contribute to the destruction of the mucosal basement membrane observed in some regions of the nasal mucosa in patients with CRSsNP, since the basement membrane is primarily formed from collagen IV type, which is known to be a substrate for MMP-9. Our findings are consistent with other studies that confirm the role of MMP-9 in the degradation of the nasal mucosal basement membrane components in chronic rhinosinusitis [15].

It has been reported that MMP-9 is involved in EMT induction [16, 17]. Lin CY et al have demonstrated that MMP-9 is able to induce EMT via participation of the Snail transcription factor [17]. Moreover, Bai X et al have shown that recombinant MMP-9 treatment increases the rate of EMT in esophageal squamous carcinoma cells [16]. Thus, we can presume that the increased number of mesenchymal-like vimentin-expressing cells found in the nasal epithelium of patients with CRSsNP, which can be considered to be the EMT induction, may be partially due to overactivation of MMP-9.

Conflict of interests

The authors declare no conflict of interest.

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