Rīgas Stradiņa universitāte

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THE ROLE OF NEUROPEPTIDES AND NEUROGENIC INFLAMMATION IN THE ISOLATED POSTNASAL DRIP SYNDROME

Summary of the Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Speciality – Otorhinolaryngology

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The Doctoral Thesis is available in the RSU library of and at RSU webpage: www.rsu.lv.

Secretary of the Doctoral Council:

Dr. med., Professor **Juta Kroiča**

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ABBREVATIONS USED

Abbrevation	English	Latvian
CGRA	Chromogranin A	Hromogranīns A
CGRP	Calcitonine gene related peptide	Kalcitonīna gēnam pradniecīgais peptīds
DT	Computer tomography	Datortomogrāfija
H&E	Haematoxylin- eosin	Hematoksilīns un eozīns
ENT	Ear, nose, throat specialist	Ausu, kakla, deguna ārsts
IgA	Immunoglobulin A	Imūnglobulīns A
IgE	Immunoglobulin E	Imūnglobulīns E
IgG	Immunoglobulin G	Imūnglobulīns G
IgM	Immunoglobulin M	Imūnglobulīns M
Il-6	Interleukin 6	Interleikīns 6
Il-10	Interleukin 10	Interleikīns 10
MMP-2	Matrix metalloprothesase 2	Matrices metaloproteāze 2
MMP-9	Matrix metalloprothesase 9	Matrices metaloproteāze 9
NPY	Neuropeptide tyrosine	Neiropeptīds tirozīns
NFkB	Nuclear factor kappa beta	Nukleārais faktors kapa beta
PGP 9.5	Proteine gene product 9.5	Proteīna gēna produkts 9,5
RSU	Rīga Stradiņš University	Rīgas Stradiņa universitāte
SP	Substance P	P viela
TNFα	Tumor necrosis factor alpha	Tumora nekrozes faktors alfa
VIP	Vasoactive intestinale polypeptide	Vazoaktīvais intestinālais polipeptīds

INTRODUCTION

Mucus discharges in the nasopharynx is a common symptom of many diseases like sinusitis, allergic rhinitis, reflux disease. There are some patients who have been excluded for those diseases, but they still have mucus discharges in the nasopharynx, a foreign body sensation in the throat and a dry cough caused by the desire to get rid of the mucus in the nasopharynx. By the examination of a physician, mucous discharges in the nasopharynx or so called post nasal drip are confirmed. This is so-called isolated postnasal drip syndrome (Sanu, 2008).

Up to now, there were some attempts to explain this syndrome with anatomical factors of the nasal cavity, like two holes syndrome. A publication of Croatian researchers poses the source of mucus discharge in the nasopharynx in the case of this syndrome is an additional hole in the posterior part of processus uncinatus, which allows mucus from maxillary sinus to flow directly to the nasopharynx explaining (Mladina, 2010). Still, there are much more questions about the fact that increased mucus formation is absent in the nasal cavity but nasopharynx.

Though this syndrome is not a life-threatening one, there still is an impairment of the life quality of patients, even more, because of unsatisfied treatment ability caused by poor understanding of underlying etiology and pathogenesis of this syndrome. In most of the cases, the leading suggestion from a physician to the isolated post nasal drip syndrome patient is to resign symptoms (Sanu, 2008).

There is a need for further studies of this syndrome taking into an account the mucosal inflammation of the nose and nasopharynx and possibility about the place of this condition localized in the nasopharynx, not the nasal cavity.

The aim of the study was to identify the role of neuroendocrine innervation and neurogenic inflammation in the nasal and nasopharyngeal mucosa, based on the level of neuropeptides, cytokines, apopthosis and tissue remodellation markers of the mucosa.

Scientific hypothesis of the study

Patients with isolated post nasal drip syndrome have a chronical mucosal inflammation in the nasal and nasopharyngeal mucosa confirmed by routine histological picture of mucosal tissue.

Patients with isolated post nasal drip syndrome have changes in the neuropeptide levels, neuroendocrine innervation of nasal and nasopharyngeal mucosa, activation of cytocines and interaction between sympathetic and parasymphatetic nervous system.

Objectives of the study

1. To analyze the morphological picture of the nasal and nasopharyngeal mucosa of the control and postnasal drip syndrome group based on haematoxyline&eosine staining.
2. To analyze the total neuroendocrine innervation and precursors of the neuroendocrine innervation in the nasal and nasopharyngeal mucosa of the control and postnasal drip syndrome patients according to the relative amount of PGP 9.5 and chromograninA immunopositive structures.
3. To analyze the involvement of the sensory, parasympathetic and sympathetic innervation of the control and postnasal syndrome patients based on the relative amount of CGRP, SP, VIP, NPY immunopositive structures in the nasal and nasopharyngeal mucosa.
4. To analyze the degeneration and remodulation of the nasal and nasopharyngeal mucosa based on the relative amount of MMP-2, MMP-9, laminin fibronectin and collagen IV immunopositive structures of the control and the postnasal drip syndrome patients.

5. To analyze the apoptosis based on the relative amount of caspase immunopositive structures in the nasal and nasopharyngeal mucosa of the control and postnasal drip syndrome patients.

6. To analyze the distribution of inflammatory and anti-inflammatory cytokines in the control and postnasal drip patients based on the relative amount of the Il-6, Il-10, TNF-a and NFkB immunopositive structures in the nasal and nasopharyngeal mucosa.

7. To analyze the involvement of the local immune system in the control and the postnasal syndrome group patients based on the relative amount of b-defensin immunopositive structures in the nasal and nasopharyngeal mucosa.

8. To perform the statistical analysis and correlation of the factors mentioned above between the control and postnasal drip patients and between the nasal and nasopharyngeal mucosa in each group.

Novelty of the study

Up to now there are no studies analyzing processes in the nasal and nasopharyngeal mucosa accoding to local changes of neuroendocrine innervation, neuropeptide and cytokine level, apoptosis and tissue remodellation.

Personal contribution

The author of this research has taken part of carrying out all stages of this research by her own. The author has examinated all of subjects involved in the study, has taken all of biopsis from both patients and control, has analysed all of ruotine histological and immunochemical evalution of tissue samples and is the author of all microphotographs.

Ethical aspects

The study has been based of the Helsinki principes of Medical Ethic and has got the approval of the Ethical comitee of Rīga Stradiņš University 2010, No. E-9 (2); 02.09.2010.

The structure and volume of the study

The doctoral thesis is written in latvian. The volume of the doctoral thesis is 158 pages including pictures, tabeles and attachments. The structure of the docoral thesis is formed in the classical thesis manner and is based on the instructions of Rīga Stradiņš University and the Cabinet Regulation No. 1001.

1. MATERIAL AND METHODS

1.1. There were two groups made:

- The target group was made from 20 patients with the isolated postnasal drip syndrome. The isolated postnasal drip syndrome was confirmed by the ENT specialist. The allergy, sinus diseases, gastroesophageal reflux disease, were excluded.
- The control group was made from 20 volunteers undergoing elective nasal septum surgery according to impaired nasal breading. The isolated postnasal drip syndrome, the allergy, and the gastroesophageal reflux disease were excluded.

1.2. The isolated post nasal drip syndrome group

1.2.1. The inclusion criteria for the isolated post nasal syndrome group was:

1. The complaints about mucus discharge in the nasopharynx at least for six months up to now.
2. Mucus discharges are visible due to examination with the naso pharyngoscope.
3. Computer tomography reveals no pathological changes in the nasal cavity nor paranasal sinuses like cysts, polyps or hyperplasia of the mucosa.
4. Allergy is excluded by allergologist performing skin prick tests and examining common and specific IgE in the peripheric blood.
5. Gastroesophageal reflux disease was excluded by the gastroenterologist.

1.2.2. The exclusion criteria for the isolated post nasal syndrome group was:

1. Any confirmed allergy.
2. Any pathological changes in the computer tomography of the nose and paranasal sinuses like mucosal hyperplasia, cysts or polyps.
3. Gastroesophageal reflux disease.

In the isolated postnasal syndrome group was made from Pauls Stradins Clinical University Hospital outpatients. They were sent to the University hospital because of unsuccessful treatment of postnasal drip. All of them followed inclusion criteria. Characteristic of those patients is visible in Table 1.1. The biopsies frop the isolated postnasal drip syndrome patient's group were taken under local anesthesia during an outpatient consultation.

Table 1.1.

The isolated post nasal drip syndrome group

No	Gender	Age	Duration of the postnasal drip	Previous treatment
1	Female	34	More than two years	Topical steroids, an antihistamine
2	Male	28	One year	Topical decongestants, topical steroids, omeprazole 20 mg twice per day
3	Female	33	Two years	Topical decongestants, topical steroids.
4	Male	50	Three years	Omeprazole 20 mg twice per day, topical decongestants, topical steroids
5	Female	54	Three years	Omeprazole 20 mg twice per day, topical steroids, an antihistamine
6	Female	59	Two years	Omeprazole 20 mg twice per day, topical steroids
7	Female	59	Four years	Topical decongestants, topical steroids
8	Male	51	More than five years	2008. rhinoseptoplasty was made; topical decongestants, topical steroids

Table 1.1. continued

No	Gender	Age	Duration of the postnasal drip	Previous treatment
9	Female	28	More than three years	Omeprazole 20 mg twice per day, topical decongestants, topical steroids
10	Female	64	More than five years	Omeprazole 20 mg daily, topical steroids
11	Female	52	More than two years	Topical steroids
12	Female	25	One year	Topical steroids
13	Female	43	Three years	Topical steroids
14	Male	53	More than two years	Topical steroids, an antihistmine
15	Female	54	Three years	Topical decongestants, topical steroids
16	Male	37	More than two years	Omeprazole 20 mg daily, topical steroids
17	Female	40	More than one year	Topical steroids
18	Female	49	More than three years	Omeprazole 20 mg twice per day, topical steroids
19	Female	64	More than five years	Topical decongestants, topical steroids, omeprazole 20 mg twice per day
20	Female	50	Four years	Topical steroids, omeprazole 20 mg per day

1.3. The control group

The control group was made from volunteers undergoing elected rhinoseptoplasty because of impaired nasal breading. The majority of patients (11) have impaired nasal breathing after the nasal trauma, the other nine patients denied any trauma in the past.

1.3.1. The inclusion criteria for the control group was:

1. The person at the moment has'nt got any complaints about postnasal drip and never had one.
2. Computer tomography of the nose and paranasal sinuses doesn't reveal any pathology like hyperplasia of the mucosa, cysts, polyps, but deviated nasal septum.
3. Negative history of acute sinusitis in the past three months.
4. Excluded allergies.
5. Excluded gastroesophagal reflux disease.

1.3.2. The exclusion criteria for the control group was:

1. Hyperplasia of the nasal mucosa
2. An acute or a chronic sinusitis
3. Gastroesophagal reflux disease
4. Any kind of allergies.

The description of the control group patients is summarised in the Table 1.2.

Table 1.2.

The control group

No	Gender	Age	Duration of the postnasal drip	Treatment
1	Female	36	No postnasal drip	Fluticasone propionate 100 µg daily because of nasal obstruction
2	Male	28	No postnasal drip	*Xylometazolin* 0.1% many times per day because of nasal obstruction
3	Male	18	No postnasal drip	None
4	Female	25	No postnasal drip	None

Table 1.2. continued

No	Gender	Age	Duration of the postnasal drip	Treatment
5	Female	40	No postnasal drip	None
6	Male	34	No postnasal drip	*Xylometazolin* 0.1% many times per day because of nasal obstruction
7	Female	42	No postnasal drip	Fluticasone propionate 100 µg daily because of nasal obstruction
8	Female	28	No postnasal drip	None
9	Male	40	No postnasal drip	*Xylometazolin* 0.1% many times per day because of nasal obstruction
10	Male	22	No postnasal drip	None
11	Male	51	No postnasal drip	Fluticasone propionate 100 µg daily because of nasal obstruction
12	Female	31	No postnasal drip	Fluticasone propionate 100 µg daily because of nasal obstruction
13	Male	39	No postnasal drip	None
14	Male	43	No postnasal drip	None
15	Male	28	No postnasal drip	*Xylometazolin* 0.1% many times per day because of nasal obstruction
16	Male	26	No postnasal drip	None
17	Male	38	No postnasal drip	None
18	Male	48	No postnasal drip	None
19	Male	43	No postnasal drip	None
20	Male	25	No postnasal drip	Fluticasone proprionate 100 µg daily because of nasal obstruction

The people from both groups participated in the study according to the free will of them, signed the agreement on the participation in the study. All the personal data from the participants of the study was coded for protection.

1.4. Investigation methods

1.4.1. The biopsies

The biopsies were made in the same way for both groups in Pauls Stradins Clinical University Hospital. From the isolated postnasal group syndrome patients biopsies were taken under local anesthesia, on the contrary under general anesthesia before the rhinoseptoplasty from the control group. The place the tissue samples were taken from were always the same: the medial part of the inferior nasal turbinate on the right side and the medial part of the nasopharyngeal arch area avoiding lymphatic tissue of pharyngeal tonsil. Under the control of Nasopharyngoscope with small biopsy forceps, nasal and nasopharyngeal mucosa tissue samples, in the average 1–2 mm diameter, were taken. Immediately there were fixated in the previous saturated Stefanini solution (2% formaldehyde, 0.2%pikrin acid, 0.1M phosphate buffer pH 7.2) (Erjelfält, 1995). In 72 hours, tissue was transported to the Department of Morphology of the Institute of Anatomy and Anthropology of Rīga Stradiņš University for the further processing.

1.4.2. The staining for the routine hystological examination

The previously fixated tissue samples in the Morphology Laboratory of the Institute of Anatomy and Anthropology were dehydrated and embedded in the paraffin. The paraffin blocks were cut 5 µm thick and stained with hematoxylin and eosin (Fisher, 2008). At the beginning deparaffinization of the

tissue was made. After the staining with hematoxylin (Mayers Hematoxylin, Bio-optical, Italy; code: 05M06002) was done for seven minutes. After the staining, tissue samples were washed in water for ten minutes and then stained with eosin (Eosin Y Alcoholic Solution, Bio-optical, Italy; code: 05B1003). Then, after a short washing with water for two – three minutes, tissue samples were dehydrated using 70° alcohol and then 90° alcohol for five minutes. Then tissue samples were covered with carboxylol for ten minutes, and after with xylol and polystirol. In the end, the coverslip for each tissue sample was used. In the result, the basophilic tissue sections were stained in the blue, but acidophilic sections, in the pink (Fisher, 2008). Slides were examined under Leica light microscope (Leica DM RB; Germany), and microphotographs were taken using Leica digital camera (Leica Microsystem AG; Germany).

1.4.3. Immunochemistry staining: Biotin- Streptavidin Method

The tissue samples from nasal and nasopharyngeal mucosa were immediately fixated in the Stefanini solution. In the Morphology Laboratory of the Institute of Anatomy and Anthropology of Riga Stradiņš University tissue samples were dehydrated and poured in paraffin blocks as described above. 3–5 μm thick sections were prepared. Further tissue processing was done according to the fallowing protocol (Hsu et al., 1981):

- Deparaffinization;
- Tissue rinsing with water and alcohol;
- Rinsing for ten minutes in the TRIS buffer (Lot 0713513, Diapath S.p.A., Italy);
- Boiling tissue with EDTA buffer (Dispatch S.p.A, Italy, code: 0713311) in a microwave oven for five minutes;

- After cooling dawn, tissue samples are rinsed twice for five minutes in the TRIS buffer;
- Tissue samples are processed with 3% peroxide for ten minutes;
- Washing in the distilled water and then in the TRIS buffer twice for five minutes;
- Minimizing the background colors with the blocking serum for 20 minutes;
- Staining with primary antibodies for one hour (Table 1.3.);
- Rinsing tissue samples in the TRSI buffer for ten minutes;
- Tissue samples staining with biotin associated secondary antibodies (DakoCytomation, Denmark; code: K1015) for 30 minutes;
- Rinsing tissue samples in the TRIS buffer for five minutes;
- Processing tissue samples with enzyme peroxidase bounded streptavidin (DakoCytomation, Denmark, code: K0690);
- Rinsing tissue samples in the TRIS buffer for five minutes;
- Processing tissue samples with DAB+ substrate chromogenic system (DakoCytomation, Denmark, code: K3468) for ten minutes achieving the brown color of immunopositive structures;
- Washing tissue samples in water;
- Staining tissue samples with hematoxylin (Mayers Hematoxylin, Bio optical, code: 05M06002) for two minutes.

The antibodies used in our study is visible in the Table 1.3.

Table 1.3.

Antibodies used in the immunochemical staining

Factor	Code	Obtained from	Working dilution	Producer
PGP 9.5	Z5116	Rabbit	1:600	DAKO (Denmark)
CGRA	A0430	Rabbit	1:400	DAKO (Denmark)
NPY	B48-100	Rabbit	1:10	DAKO (Denmark)

Table 1.3. continued

Factor	Code	Obtained from	Working dilution	Producer
VIP	Ab22736	Rabbit	1:400	Abcam (UK)
caspasis	Ab52951	Mouse	1:100	Abcam (UK)
MMP-2	AF902	Goat	1:100	RD Systems (Germany)
MMP-9	AF902	Rabbit	1:250	RD Systems (Germany)
Il-6	Sc-73319	Mouse	1:100	Santa Cruz Biotechnology (USA)
Il-10	Ab-34843	Rabbit	1:400	Abacam (UK)
TNFα	P23563	Mouse	1:100	RnD Systems (Germany)
β defensin	AF2758	Goat	1:100	RnD Systems (Germany)
NFkB	Sc-109	Rabbit	1:200	Santa Cruz Biotechnology (USA)
laminin	LS-C49219	Mouse	1:100	Lifespan (Spain)
fibronectin	A0245	Rabbit	1:100	Invitrogen (USA)
collagen IV	Clone-CIV94	Mouse	1:30	Invitrogen (USA)
CGRP	281328	Rabbit	1:20	Quartet (Germany)

PGP 9.5 – proteine gene product 9.5; CGRA – chromogranin A; NPY – neuropeptide tyrosine; VIP – vasoactive intestinale polypeptide; MMP-2 – matrix metalloprothease 2; MMP-9 –matrix metalloprothease 9; Il-6 –interleukin 6; Il-10 – interleukin 10; TNF α – tumor necrosis factor α; NFkB – nuclear factor kappa beta; CGRP – calcitonine gene realted peptide

1.5. Data processing methods

The semiquantitative counting method was used for the registration of the relative amount of immunopositive structures (Pilmane, 1998). This method allows not only to count the immunopositive structures but also to reveal the localization of them, like in the stroma, epithelium, blood vessels, etc. The final mark was given after the examination of three random visual field of the tissue sample.

The denotations of the semiquantitative method and equation to numbers for statistic analysis are visible in Table 1.4.

Table 1.4

The denotations of semiquantitative counting method and equation to numbers

Mark	Equal number	Explantation
–	0	No immunopositive structures in the visual field.
0/+	0.5	Rare immunopositive structures in the visual field.
+	1	A few immunopositive structures in the visal field.
++	2	Moderate immunopositive structures in the visal field.
+++	3	Numerous immunopositive structures in the visal field.
++++	4	Abudance of immunopositive structures in the visal field.

1.6. Statistical analysis of the data

The morphological changes of the mucosal tissue were analyzed using descriptional statistical methods (*Teibe*, 2007).

Statistical analysis of the immunochemistry data was conducted using nonparametric statistical methods. We calculated the median, the 25 and 75 percentiles of each neuroendocrine innervation, the inflammatory cytokine, tissue remodellation marker and apoptosis marker included to our study. Further, we used those results to calculate the correlation between the isolated postnasal drip syndrome and the control group using Kruskal–Wallis test. The correlations of the distribution of immunochemistry markers inside the group (nasal mucosa vs. nasopharyngeal mucosa) were calculated using Spearman's Rank-Order correlation test. The p-value < 0.05 confirmed statistically significant result (*Teibe*, 2007).

The statistical calculation was done using Statistica 2.0 software (Dell, USA).

2. RESULTS

2.1. Morphological findings

In all tissue samples from both groups, all layers of upper respiratory mucosa were visible. The tissue samples from the control group showed normal respiratory mucosa made from stratified ciliated epithelium, the basal membrane, and lamina propria (Figure 2.1.). Similar findings were in the nasopharyngeal mucosa of the control group. There were no pathological changes in the nasal, nor nasopharyngeal mucosa of the control group.

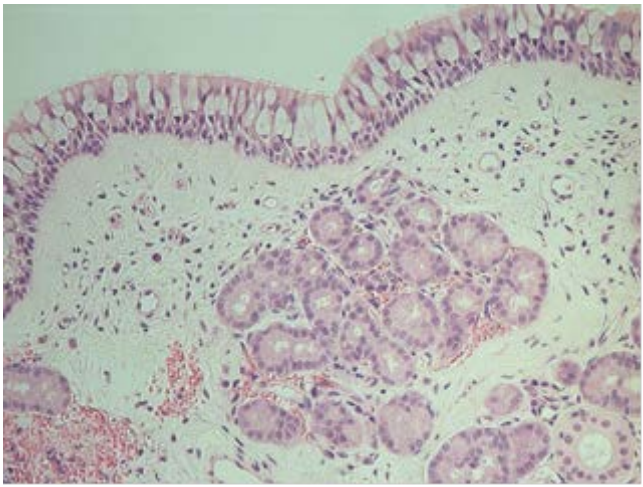

Figure 2.1. **Nasal mucosa of the control group. A stratified ciliated epithelium, well developed submucosal glands and rare lymphocytes in the *lamina propria* are visible. H&E × 200**

The isolated postnasal drip syndrome group tissue samples reveled changes in the nasal mucosa of the chronic inflammation with hyperplasia of the submucosal glands and epithelium, thickened basal membrane, infiltration of lymphocytes in the lamina propria (Figure 2.2.). In some tissue samples, sclerosis of small arterioles was found.

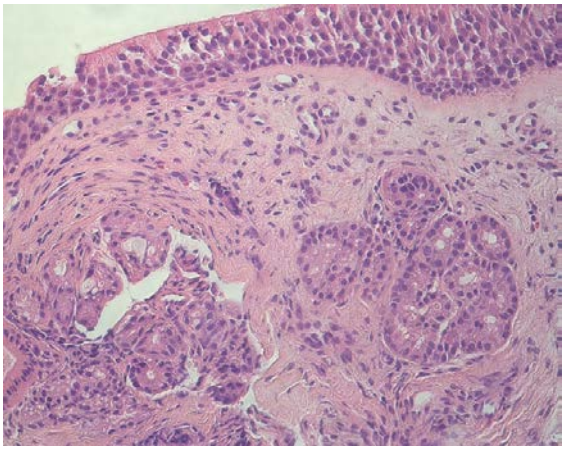

Figure 2.2. **The nasal mucosa of the isolated post nasal syndrome group was thickened basal membrane, hyperplasia of epithelium and submucosal glands, as well as infiltration of lymphocytes in the lamina propria, is visible. H&E × 200.**

The results of the routine morphological examination of the nasal and nasopharyngeal mucosa tissue of both groups are seen in Table 2.1.

Table 2.1.

The results of routine morphological examination

Morphological findings	**Nasal mucosa control**	**Nasal mucosa patients**	**Nasopharyngeal mucosa control**	**Nasopharyngeal mucosa patients**
Epithelial hyperplasia	+	++	+	+++
Thickened basal membrane	+	++	+	+++
Infiltration of lymphocytes in the *lamina propria*	+	++	+	++
Hyperplasia of submucosal glands	+	++	+	+++

+ a few immunopositive structures in the visual field; ++ moderate number of immunopositive structures in the visual field; +++ numerous immunopositive structures in the visual field; abundance of immunopositive structures in the visual field. (Pilmane, 1998).

2.2. The marker of the tissue neuroendocrine innervation protein gene product 9.5 (PGP 9.5)

The control group showed a few (+) PGP 9.5 containing structures in the nasal and nasopharyngeal mucosa. In the nasal mucosa, PGP 9.5 immunopositive nerve fibers were found in the submucosa around blood vessels and glands. Similar findings were in the nasopharyngeal mucosa of the control group (Figure 2.3.).

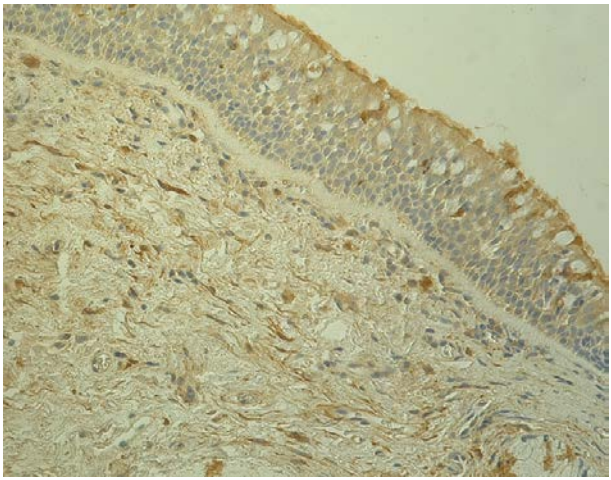

Figure 2.3. **A nasopharyngeal mucosa of the control group showing a few PGP 9.5 containing nerve fibers in the lamina propria. PGP 9.5 × 200.**

The isolated postnasal drip syndrome group showed numerous (+++) of PGP 9.5 immunopositive nerve fibers in the nasal mucosa. Mainly those PGP9.5 immunopositive structures were localized around blood vessels and submucosal glands. In the nasopharyngeal mucosa of the postnasal drip group also a numerous (+++) of PGP 9.5 containing nerve fibers next to the submucosal glands and blood vessels (Figure 2.4.).

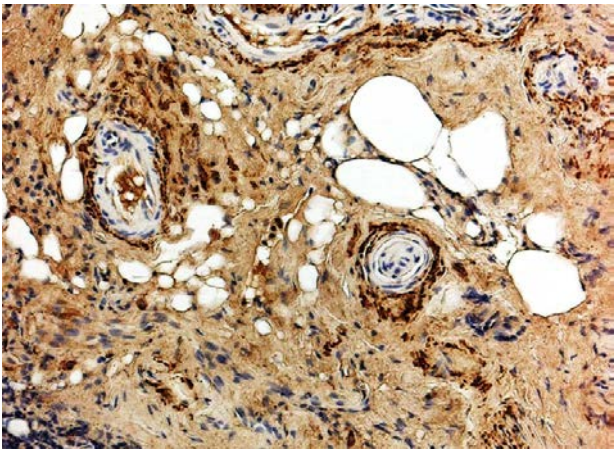

Figure 2.4. **The nasopharyngeal mucosa of the isolated postnasal drip syndrome where numerous/abundance (+++/++++) of PGP 9.5 containing nerve fibers are visible around sclerotised blood vessels and submucosal glands. PGP 9.5 ×200**

There were statistically significant increasing of PGP 9.5 containing nerve fibers in the isolated postnasal syndrome group (Kruskal–Wallis test, $p<0.0001$).

There were no statistically significant changes found between nasal and nasopharyngeal mucosa according to the PGP 9.5 containing fibers in the control group (Spearman's Rank-Order correlation test $p=0.528757$).

The isolated postnasal drip syndrome group reveled statistically significant increasing of PGP 9.5 containing structures in the nasopharyngeal mucosa (Spearman's Rank-Order correlation test $p=0.002966$).

2.3. Chromogranin A (CGRA)

The tissue samples from the control group's nasal mucosa showed a few (+) CGRA immunopositive granules containing cells in the epithelial layer of submucosal glands. Nasopharyngeal mucosa samples of the control group showed similar findings; there were a few (+) CGRA containing cells in the

visual field, mainly in the submucosal glands. Thre were no statistically significant differences in the CGRA distribution in the nasal and nasopharyngeal mucosa (Spearman's Rank-Order Correlation test p=0.5287).

The isolated post nasal syndrome patient's nasal mucosa showed a moderate (++) number of CGRA containing cells which were localized in epithelium and submucosal glands. The nasopharyngeal mucosa samples of the isolated postnasal drip group showed numerous (+++) CGRA immunopositive cells localized next to the sclerotized blood vessels (Figure 2.5.). There were no statistically significant changes found in the distribution of the CGRA containing cells of isolated post nasal group patients (Spearman's Rank-Order correlation test p=0.062772).

Kruskal-Wallis test confirmed statistically significantly the higer amount of CGRA containing cells in the isolated postnasal drip syndrome group ($p<0.0001$).

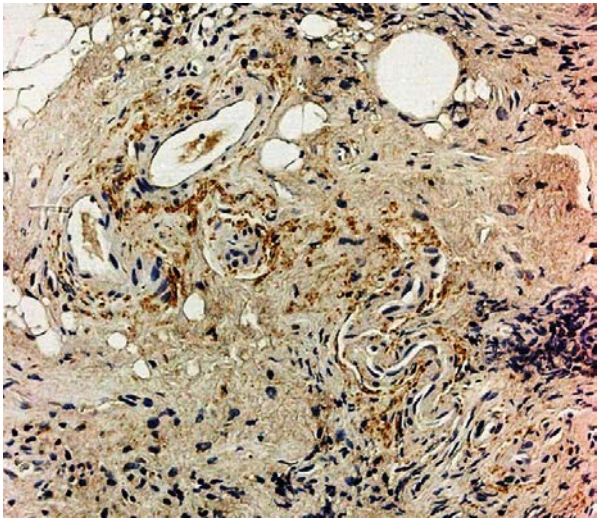

Figure 2.5. **Isolated post nasal drip syndrome patient's nasopharyngeal mucosa with numerous (+++) CGRA containing cells around sclerotized blood vessels. CGRA × 200**

2.4. Vasoactive intestinal polypeptide (VIP)

The nasal mucosa of the control group showed rare (0/+) VIP immunopositive fibers in the visual field. The nasopharyngeal mucosa of the control group showed a few (+) VIP-containing nerve fibers in the visual field.They were mainly localized around the submucosal blood vessel. There were no statistically significant differences between nasal and nasopharyngeal mucosa found (Spearman's Rank-Order correlation test p=0.299690).

The nasal mucosa of the isolated post nasal drip syndrome group showed a moderate number (++) of VIP-containing nerve fibers in the visual field; mainly they were localized around blood vessels, but vas found next to the submucosal glands as well. The nasopharyngeal mucosa of isolated post nasal drip syndrome patients reveled a moderate number of VIP immunopositive nerve fibers around submucosal blood vessels (Figure 2.6.).

There were no statistically significant differences in the VIP distribution between nasal and nasopharyngeal mucosa found (Spearman's Rank-Order correlation test p=0.608158). However, Kruskal–Wallis test confirmed statistically significantly the biger amount of VIP-containing structures in the isolates post nasal drip syndrome patients ($p<0.0004$).

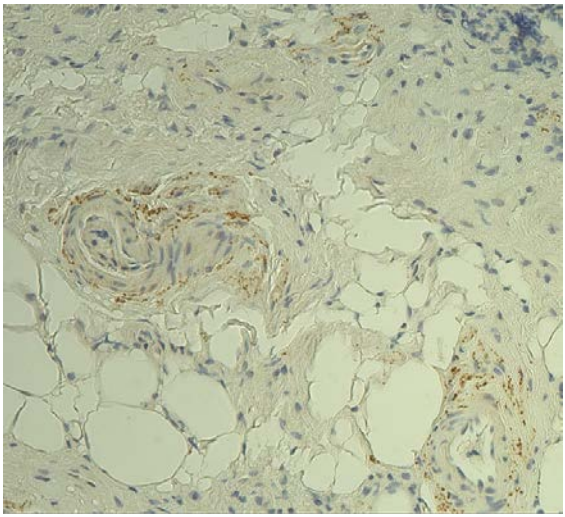

Figure 2.6. **Isolated post nasal drip syndrome patient's nasal mucosa showing numerous VIP-containing nerve fibers around blood vessels. VIP × 200**

2.5. Neuropeptide Y (NPY)

The nasal mucosa of the control group contained only rare (0/+) NPY immunopositive structures in the visual field. The nasopharyngeal mucosa of the control group showed a few (+) NPY containing nerve fibers around submucosal glands. Spearman's Rank-Order Correlation test showed no statistically significant differences between nasal and nasopharyngeal mucosa of the control group (P=0.2996).

The nasal mucosa of isolated post nasal drip syndrome groups revealed a few NPY immunopositive nerve fibers around submucosal glands. The nasopharyngeal mucosa showed a moderate number of NPY immunopositive fibers next to the submucosal glands and blood vessels. Using Spearman's Rank-Order Correlation test we haven't found statistically significant changes in the nasal and nasopharyngeal mucosa (p=0.15). The statistically significantly

higher amount of NPY immunopositive structures were found in the isolated post nasal syndrome group (Kruskal–Wallis test $p<0.0001$).

2.6. Substance P (SP)

The nasal mucosa of the control group showed just rare (0/+) SP containing structures in the visual field. Similar findings revealed nasopharyngeal mucosa of the control group, just rare (0/+) SP immunopositive nerve fibers in the visual field.

The isolated postnasal drip syndrome patients nasal and nasopharyngeal mucosa showed rare (0/+) SP immunopositive nerve fibers in the visual field.

There was no statistically significant difference in the SP distribution between both groups.

2.7. Calcitonin gene-related peptide (CGRP)

The nasal and nasopharyngeal mucosa of the control group showed just rare (0/+) CGRP immunopositive structures in the visual field, localized around submucosal glands.

The nasal and nasopharyngeal mucosa of isolated postnasal drip syndrome patients revealed just rare (0/+) CGRP immunopositive nerve fibers in the visual field. The CGRP-containing structures were found next to the submucosal glands.

There were no statistically significant differences between the control and the isolated postnasal drip syndrome group in the distribution of CGRP.

2.8. Caspase (the marker of apoptosis)

In the nasal mucosa of the control group a few (+) caspase immunopositive cells are found, localized in the epithelium and submucosal glands. In the nasopharyngeal mucosa, we found a moderate number (++) of caspase immunopositive cells localized in the submucosal glands. Spearman Rank-Order Correlation test didn't reveal the statistically significant difference between the nasal and nasopharyngeal mucosa of control group patients ($p<0.647447$).

The isolated postnasal drip syndrome patients nasal mucosa contained a moderate number (++) of caspase immunopositive cells, mainly they were localized in the epithelium, glands and the lamina propria around blood vessels (Figure 2.7).

In the nasopharyngeal mucosa of isolated postnasal drip group, we found numerous (+++) caspase immunopositive cells in the epithelium, lamina propria, and submucosal glands. Spearman's Rank-Order correlation test confirmed a higher amount of caspase- containing cells in the nasopharyngeal mucosa of patients ($p<0.008553$).

There were statistically significantly the higer amount of caspase-containing cells in patients group by comparing to the control group. Kruskal–Wallis test $p<0.0003$ was for the nasal mucosa and $p<0.0001$ for the nasopharyngeal mucosa.

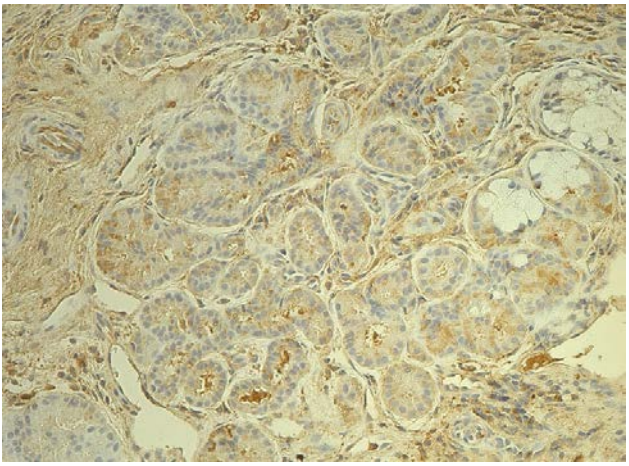

Figure 2.7. **The nasal mucosa of isolated postnasal drip patient, where moderate/numerous (++/+++) caspase-containing cells are visible into the stroma and submucosal glands. Caspase × 200**

2.9. The markers of tissue remodellation- matrix metalloproteases

2.9.1. Matrix metalloprotease 2 (MMP-2)

The nasal and nasopharyngeal mucosa of the control group revealed rare (0/+) MMP-2- containing structures in the *lamina propria*. Spearman's Rank-Order correlation test didn't reveal a difference between nasal and nasopharyngeal mucosa of the control group ($p<0.54$).

The nasal and nasopharyngeal mucosa of the isolated postnasal drip syndrome group showed rare (0/+) MMP-2-containing cells in the lamina propria. Spearman's Rank-Order correlation test didn't reveal a difference between nasal and nasopharyngeal mucosa of the postnasal drip syndrome group ($p<0.5$).

There were no statistically significant differences between the control ant the postnasal drip syndrome group in MMP-2 distribution.

2.9.2. Matrix metalloprotease 9 (MMP-9)

The nasal mucosa of the control group showed a few/a moderate (+/++) number of MMP-9-containing cells in the *lamina propria* next to the basal membrane. Spearman's Rank-Order correlation test didn't reveal a difference between nasal and nasopharyngeal mucosa of the control group.

The nasal mucosa of isolated postnasal drip syndrome showed numerous (+++) MMP-9 immunopositive cells in the epithelium and *lamina propria*. The nasopharyngeal mucosa of postnasal drip patients showed numerous (+++) MMP-9 immunopositive cells in *lamina propria*, submucosal glands, and epithelium (Figure 2.8.). Spearman's Rank-Order correlation test didn't reveal a difference between nasal and nasopharyngeal mucosa of the control group.

Kruskal–Wallis test confirmed the higer amount of MMP-9- containing cells in the isolated postnasal drip syndrome patients nasal and nasopharyngeal mucosa.

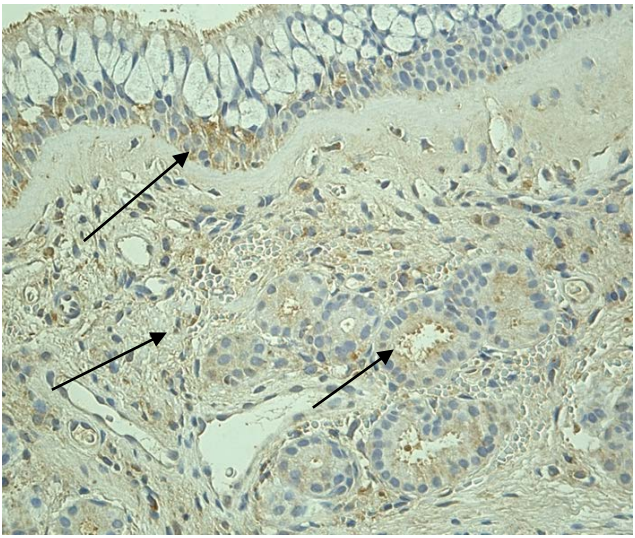

Figure 2.8. **The nasopharyngeal mucosa of isolated postnasal drip syndrome patients showing numerous (+++) MMP-9-containing cells in the submucosal glands, epithelium and *lamina propria* (arrows). MMP-9 × 200**

2.10. Laminin

Both, the control and the isolated postnasal drip syndrome mucosal tissue were laminin negative.

2.11. Collagen IV

There was rare (0/+) collagen IV − containing structures in the nasal and nasopharyngeal mucosa of the control group.

There was only rare (0/+) collagen IV − containing fibers in the isolated postnasal drip syndrome patients nasal and nasopharyngeal mucosa. There weren't any statistically significant differences between the control and the isolated postnasal drip syndrome group in collagen IV distribution.

Kruskal–Wallis test confirmed statistically sifnificant increasing in fibronectin immunopositive fibers in the isolated post nasal drip syndrome patients nasal ($p<0.0001$) and nasopharyngeal ($p=0.0001$) mucosa.

2.12. Fibronectin

The nasal mucosa of the control group contained a moderate (++) number of fibronectin immunopositive fiber in lamina propria. The nasopharyngeal mucosa of the control group also showed a moderate number (++) of fibronectin immunopositive fibers in the *lamina propria*. Spearman's Rank-Order correlation test didn't reveal statistically significant difference between nasal and nasopharyngeal mucosa in the fibronectin distribution ($p=0.372839$).

The isolated postnasal drip syndrome patients nasal mucosa contained a moderate (++) number of fibronectin immunopositive fibers in the *lamina propria* (Figure 2.9.), but nasopharyngeal mucosa of this group contained

numerous (+++) fibronectin immunopositive fibers in the *lamina propria*. The Spearman Rank-Order Correlation test didn't reveal statistically significant difference between nasal and nasopharyngeal mucosa in the fibronectin distribution (p=0.114).

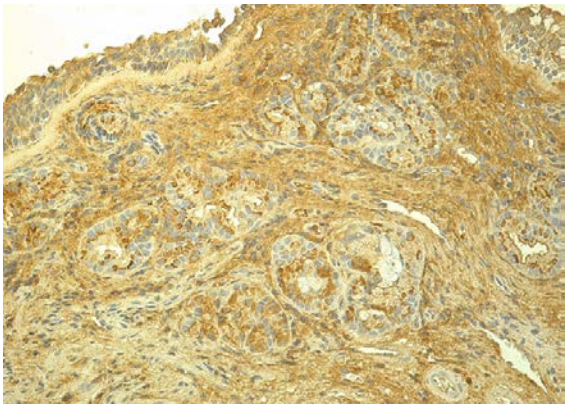

Figure 2.9. **The nasal mucosa of isolated postnasal drip patient with abundance fibronectin immunopositive fibers in the *lamina propria*. Fibronectin × 200**

2.13. Markers of the inflammation

2.13.1. Interleukin 10 (Il-10)

The nasal mucosa of the control group showed rare (0/+) Il-10 immunopositive structure in the visual field. Similar finding showed the nasopharyngeal mucosa of the control group, just rare (0/+) Il-10 immunopositive structure next to the submucosal glands. There was no statistically significant difference between the nasal and the nasopharyngeal mucosa in Il-10 distribution (Spearman's Rank-Order correlation test p=0,744769).

The isolated postnasal drip syndrome patient's nasal mucosa showed a moderate number (++) of Il-10 immunopositive cells in the lamina propria around blood vessels. The nasopharyngeal mucosa of isolated post nasal drip

syndrome patients showed moderate number (++) of Il-10 containing cells in the *lamina propria* next to blood vessels (Figure 2.10). Spearman's Rank-Order correlation test confirmed higher amount of Il-10 containing cells in the nasopharyngeal mucosa of isolated postnasal drip syndrome patient ($p=0.0346$).

Kruskal–Wallis test was used to confirm statistically significant increase of Il-10 containing cells in the patients nasal and nasopharyngeal mucosa ($p<0.0001$).

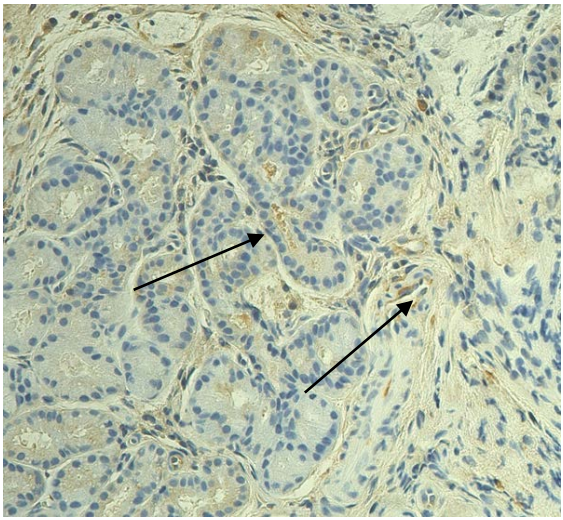

Figure 2.10. **The nasopharyngeal mucosa of isolated postnasal drip syndrome patient showing a few (+) Il-10 containing cells in the *lamina propria* and glands (arrows).** Il-10 × 200

2.13.2. Interleukin 6 (Il-6)

The controls group nasal, and nasopharyngeal mucosa showed just rare (0/+) Il-6 containing cells in the visual field next to submucosal glands. There was no statistically significant difference between the nasal and the nasopharyngeal mucosa in Il-6 distribution (Spearman's Rank-Order correlation test $p=0.5$).

The nasal mucosa of patients group revealed moderate (++) number of Il-6-containing cells next to submucosal glands. The nasopharyngeal mucosa of isolated postnasal drip syndrome revealed numerous (+++) Il-6-containing cells in the submucosal glands. Spearman's Rank-Order correlation test didn't reveal statistically significant differences between nasal and nasopharyngeal mucosa ($p=0.254323$). By compeering both groups statistically significant increase of Il-6-containiing cells was fund in isolated postnasal drip syndrome patients (Kruskal–Wallis test $p<0.0001$).

2.13.3. Nuclear factor kB (NFkB)

In the controls group nasal and nasopharyngeal mucosa only rare (0/+) NFkB immunopositive structure were found. Not all of controls goups tissule samples contained NFkB immunupositives structures. Spearman's Rank-Order correlation test didn't reveal statistically significant differences between nasal and nasopharyngeal mucosa ($p=0.416$).

The isolated postnasal drip syndrome patients nasal mucosa containied a few (+) NFkB immunopositive cells localized next to submucosal glands and blood vessels. The nasopharyngeal mucosa contained a moderate (++) number of NFkB immunopositive cells in the visual field, localized next to submucosal glands and blood vessels (Figure 2.11.) Spearman's Rank-Order correlation test didn't reveal statistically significant differences between nasal and nasopharyngeal mucosa ($p=0.062$). By compeering both groups statistically significant increase of NFkB containiing cells was fund in isolated postnasal drip syndrome patients nasal and nasopharyngeal mucosa (Kruskal–Wallis test $p<0.0001$).

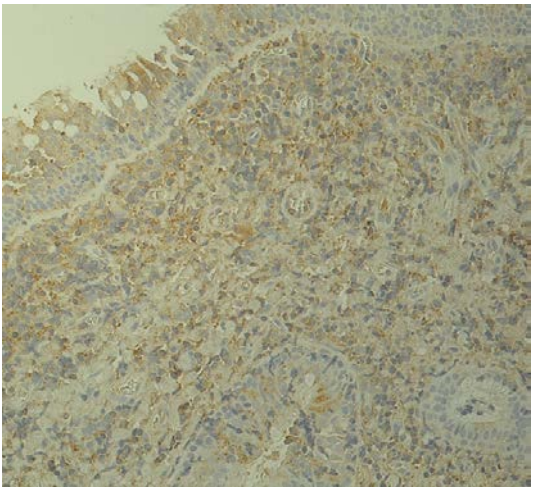

Figure 2.11. **The nasopharyngeal mucosa of isolated postnasal drip patient with numerous (+++) NFkB-containing cells is visible. NFkB × 200**

2.13.4. Tumor necrosis factor α (TNFα)

There were no TNFα immunopositive cells found in the controls group nasal mucosa. In the nasopharyngeal mucosa of the control group, just rare (+)TNFα immunopositive structure were found. Spearman's Rank-Order correlation test didn't reveal the difference between the nasal and nasopharyngeal mucosa of the control group ($p=0.3353$).

The isolated postnasal drip syndrome group revealed a few (+) TNFα immunopositive cells in submucosal glands. The nasopharyngeal mucosa if this group revealed a few (+)TNFα immunopositive cells in the visual field, mainly localized in submucosal glands. Spearman's Rank-Order correlation test didn't reveal the difference between the nasal and nasopharyngeal mucosa of the patients group ($p=0.2199$). Compeering both groups using Kruskal–Wallis test, found statistically significant increase of TNFα immunopositive cells in the patients nasal and nasopharyngeal mucosa ($p<0.0001$).

2.14. β defensin

In the nasal mucosa of the control group, no β defensin- containing structures were found. In the nasopharyngeal mucosa of the control group, we found a few β defensin-containing cells in the visual field. No differences in the distribution of β defensin containing cells were found (Spearman's Rank-Order correlation test p=0.217). The nasal mucosa of isolated postnasal drip syndrome patients contained a few β defensin immunopositive cells in the basal layer of epithelium and submucosal glands. In the nasopharyngeal mucosa of postnasal drip patients, a moderate number of β defensin immunopositive cells were found (Figure 2.12.). Spearman's Rank-Order correlation test didn't reveal a difference between nasal and nasopharyngeal mucosa of the control group(p= 0.153463).

Compeering both groups, statistically significant increase of β defensin containing cells was found in the isolated postnasal drip syndrome patients nasal (p=0.0017) and nasopharyngeal (p=0.000003) mucosa.

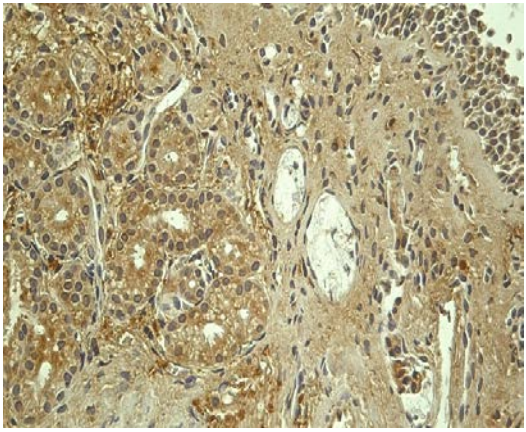

Figure 2.12. **The nasal mucosa of the isolated postnasal drip patient shows moderate/numerous β defensin containing cells in the lamina propria and submucosal glands. β defensin × 250**

3. DISCUSSION

Postnasal drip or mucus discharges in the nasopharynx is a common symptom in the case of sinusitis, allergy, gastroesophageal disease (Fokkens, 2012). Some patients still have postnasal mucus discharges with following symptoms like a foreign body sensation, irritation, and discomfort in the nasopharynx and throat, dry cough without any clinically symptomatic disease explaining it, described as isolated postnasal drip syndrome (Sanu, 2008). However postnasal drip syndrome isn't a life threatening condition,it has a significant influence on patients life quality. Hence at the moment the etiology and pathogenesis of this syndrome are not clear, there is no effective treatment for these patients. Mainly the treatment of this syndrome is based on getting rid of the symptoms at the moment and sometimes even nothing but the suggestion to put up with it (Sanu, 2008).

The aim of our study was to investigate nasal and nasopharyngeal mucosa in an aspect of tissue neuroendocrine innervation, inflammation, apoptosis and tissue remodulation. There is no similar study found in the literature. In general, there were no publications in the medical databases, describing nasal and, especially, nasopharyngeal mucosa in such aspect. There are a few studies describing the postnasal drip syndrome. It is mainly mentioned in connection with irritated chronic cough syndrome (Terasaki, 2014). Searching PubMed database by the keyword “postnasal drip syndrome,” there were only 126 publications, mainly describing postnasal drip as a symptom of some other disease. There are still many contradictions according to the etiology, pathogenesis of postnasal drip syndrome and it’s relationship with other unclear syndromes of the airways, as the chronic cough syndrome (Fokkens, 2015). And there are still controversies in the terminology between European and USA clinics. But we should admit that there is an improvement of the situation and actualisation of the postnasal drip syndrome lately

confirmed by the increasing numbers of studies and section themes in congresses.

Increased postnasal mucus discharges and complaints related to them tried to explain according to anatomical aspects of the nasal cavity and paranasal sinuses using so-called two hole syndrome (Mladina, 2010). According to the described study, the reason for postnasal discharge is the second opening in the maxillary sinus, localized in the posterior part of *processus uncinatum*. In this case, the mucus discharges from the maxillary sinus directly go to the nasopharynx causing postnasal drip. Still, according to the Mladina study, in the control group the second hole was found as well (2.2%), suggesting, similar like we do, that there is not enough with just a second hole, there should an increased production of mucus present as well in our study, we didn't examine patients looking for the second opening of the maxillary sinus, but the patient's inclusions criteria excluded patients with sinusitis and any changes in the sinuses in the CT scan.

Many studies are describing the relationship between the gastroesophageal reflux and airway disease (Velanovich, 2009; Flook, 2011), but the exact mechanism of the pathogenesis remains unclear. Probably the reason for the airway inflammation in the case of gastroesophageal reflux is not only the acid but the pepsin as well. In Our study all the patients were gastroesophageal reflux excluded, moreover, they didn't get any improvement on proton pump inhibitors therapy in the past. Ozmen (2008) described positive pepsin findings in the upper airway mucosa for patients with chronic rhinosinusitis. Still, the patients with negative pH meter had negative pepsin findings, but still, they had chronic rhinosinusitis.

The isolated postnasal drip syndrome could be taken as an idiopathic rhinitis, following Van Rijswijk (2005) criteria: the patients have complaints about nasal obstruction and/or discharges, allergy, smoking, nasal polyps, anatomical reasons, like deviated nasal septum, pregnancy or any other

hormonal conditions are excluded, and patients have no improvement on topical steroids, which ultimately meet the including criteria for our study. Unfortunately, there is no unity describing morphological changes of the idiopathic rhinitis. Some studies claiming pronounced morphological changes very similar to the allergic rhinitis like infiltrations of leucocytes and plasma cells in the nasal mucosa (Poowe, 2001). Others are describing minimal or no changes at all in the nasal mucosa in the case of idiopathic rhinitis (Van Rijswijk, 2003). Our study shows an inflammation of mucosa tissue in the case of postnasal drip with thickened basal membrane, infiltration of lymphocytes in the stroma of mucosa and hyperplasia of the epithelial layer and submucosal glands. There were no inflammation – like changes in the control group's mucosa in our study, this finding conflicts with the Kamani (2014) study, who describes lymphocyte infiltration in the mucosal stroma of lower nasal turbinate in the case of deviated nasal septum. The difference between our's and Kamani's study could be explained according to the definite inclusion/ exclusion criteria of our control group. The main exclusion criteria for the control group was any thickening of nasal mucosa as well as discharges from the nose. Kamani's study does'nt educate this aspect of the included people. One study confirms similar morphological changes (lymphocyte infiltration, hyperplasia of submucosal glands and epithelium) of nasal mucosa describing chronic rhinosinusitis (Muluk, 2004). In the control group study was made from people undergoing nasal septum surgery just like in our study, and just like in our study no pathological changes were found in the routine morphological analysis of the control group mucosa.

Vacheir (2004) found the connection between an irritator, like tobacco smoke, and nonspecific changes of the airway mucosa. This fact should be taken into the account because not only tobacco smoke but enviromental pollution could be an irritation of airways as well causing morphological and functional changes in the airway mucosa (Meggs, 1996). We found no

connections in our study between any common irritant and postnasal drip. There were smokers and nonsmokers, citizens of the major cities and country included in both groups, but no statistically significant changes in connection of behaviors and postnasal drip were found.

In the publications, the marker of neuroendocrine innervation PGP 9.5 is mainly described in connection with many olfactory conditions. In our study, we didn't analyze olfactory epithelium. There are a just a few studies relating to the neurogenic inflammation of the nasal mucosa and PGP 9.5. One of the best is the study made by Hauser-Kronberger (1997). In this study PGP 9.5, substance P and calcitonin gene-related peptide (CGRP) is described in the airway mucosa of healthy people. This study could be taken as a primary for the analysis of neuroendocrine innervation in healthy people. Findings of Hauser-Kronberger study corresponds to the control group results of our group confirming the suitability of our study design.

Figueroa (1998) describes PGP 9.5 findings in the nasal mucosa of children suffering from chronic rhinitis. The PGP 9.5 containing nerve fibers were found around submucosal glands and blood vessels. The results of this study match with our study confirming the increase of PGP 9.5 innervation in a case of mucosa inflammation.

The majority of studies analyzing PGP 9.5 in the airway mucosa are done using animal models (Forsgren, 1999; Prince 2003; Furukawa, 2008). Studies analyzing human tissue samples are less, and the count of tissue samples is very limited. This situation could be related to the growing ethical and legal claims for the use of human tissue in research. We used 20 patients and 20 control group people's tissue samples. In this aspect, our study is treasured.

Chromogranin A is a marker of neuroendocrine innervation. Chromogranin A is widely investigated and described as a marker of irritated bowel diseases and even is recommended as a diagnostic marker of this disease

(El-Salhy, 2012). In the nasal mucosa chromogranin A is used as a marker of neuroendocrine cells. Sieśkiewicz (2007) published a study analyzing different types of chronic hyperplastic rhinitis in an aspect of chromogranin A and concludes that there are no differences in the Chromogranin A distribution between different types of chronic rhinitis. On the contrary, Jornot (2008) describes the increase of Chromogranin A in airway mucosa and the positive correlation between Chromogranin A and cytokines, VIP, NPY and growth factors in the airway mucosa in the case of a chronic airway inflammation. We found statistically significant increase of chromogranin A in the postnasal drip syndrome patients nasal and nasopharyngeal mucosa. This fact suggests about the increase of neuroendocrine innervation in the case of postnasal drip syndrome and could be one of the pathogenetic keypoint of this disease.

Vasoactive intestinal polypeptide (VIP) primary shows a vasodilatation activity, but in the nasal mucosa, it is described as an activator of cilia movement and secretion of submucosal glands (Baraniuk, 1990; Lee, 2013). VIP claims the responsibility about increased mucus discharges in the airway mucosa and is described in the studies of allergic and non-allergic rhinitis (Lacroix, 1994; 1996). Groneberg (2003) publishes the study of toxic rhinitis suggesting then the most common type of a rhinitis is a toxic one. The reason for the toxic rhinitis could be ozone, nickel, chrome, tobacco smoke, etc. In this study involved patients complained about dryness in the nose, opposite situations by compeering with our patients, and the opposite was the results. Gronberg found no differences in VIP-containing structures in the nasal mucosa, on the contrary, our study shows a statistically significant increase of VIP-containing nerve fibers in the nasal mucosa. This fact confirms the possible VIP involving in the pathogenesis of postnasal drip syndrome resulting in increased mucus production in the nasopharynx.

NPY (neuropeptide Y) is a primary neuropeptide of the sympathetic nerve system ant it’s role in different airway diseases is well described.

Originally it was outlined in the animal model (Lacroix, 1994; Revington, 1997) as a vasoconstrictor and VIP antagonist. Augustyniak (2012) described NPY ability to induce immune response and phagocytosis in the nasal mucosa in a case of bacterial (*M. catarrhalis*, *H. influenzae*) infection. Our study shows significantly increased NPY immunopositive structures in the isolated postnasal drip syndrome patients mucosa suggesting about strong implication of the sympathetic nervous system in the development of postnasal drip syndrome.

Calcitonin gene-related peptide (CGRP) is localized in sensory nerve fibers, and it realizes vasodilatation in the case of neurogenic inflammation. Senanayake (2013) publishes the study confirming the release of CGRP in a case of impaired nasal breathing resulting in nasal and intracerebral vasodilatation and headaches. CGRP increase is described in the allergic rhinitis (Fischer, 2005) but decreases in the hyperplastic rhinitis (Gungor, 1999). Our study doesn't show any differences in CGRP distribution between the study and the control group. This fact doubts neurogenic inflammation and allergic reaction as a possible pathogenic mechanism of the isolated postnasal drip syndrome.

Caspase is a marker of apoptosis. Increased caspase-containing cells in the nasal mucosa are related to many diseases of the upper airway, the certain effect and relations to severity of inflammation still remains unclear. Brydon (2003) published data about increased caspase activity in a case of virus infection in an epithelial cell module. Cho (2008) described no changes in caspase-containing cells distribution between healthy nasal mucosa and nasal polyps. Further research confirmed different caspase activity in various chronic inflammation of nasal mucosa. The highest caspase activity was described in the nasal polyps and medicine induced chronic rhinitis suggesting the primary role of apoptosis in the pathogenesis of inflammation fallowing by tissue remodelation (Hirt, 2009). Trimarchi (2006) study described caspase activity in a case of Wegener’s granulomatosis and nasal polyps and hadn’t found any

statistically significant difference, but there was an increased caspase activity in a cocain abuse. Wang (2014) studied caspase activity on the cultured epithelial tissue model and found out decreasing caspase activity in the earl stages o fan inflamation and increasing the activity of caspase in a late stage of inflammation. Our study reveals moderate to numerous caspase containing cells in the nasal and nasopharyngeal mucosa of isolated postnasal drip syndrome patients confirming long term chronic inflammation with fallowing apoptosis of mucosa tissue of the nose and especially nasopharynx.

Matrix metalloproteases are markers of healing and tissue remodelation. Pure healing of tissue occurs with a little remodelation, but in a case of secondary infection, increasing of matrix metalloproteases is evident (Tansavatdi, 2010). MMP-9 is described in nasal polyps, but studies didn't reveal any correlations between severity of the nasal polyp disease, an allergy and amount of MMP containing cells in the polyp tissue (Eyibilen, 2011; Wang, 2012; Malinsky, 2013). A publication of Detwiller (2013) claims a higher amount of MMP-9 containing structures in case of nasal polyps and osteitis. In our study increased amount of MMP-9-containing cells was found in the patient's mucosa. This fact suggests about tissue remodelation process in case of isolated postnasal drip syndrome.

Liotta (1990) describes the ability of matrix metalloproteases to destroy many of the proteins localized to the basal membrane. In our study, we found negative laminin tissue in both groups and rare Collagen IV immunopositive structures. Adverse findings of those components of the basal membrane could be related to the activity of matrix metalloproteases. However, negative results were both, in the control and the study group. This fact doubts the possible intervention of basal membrane proteins in the pathogenesis of postnasal drip.

Fibronectin is also related to the tissue remodeling process. Fibronectin takes a part in a tissue healing, cellular adhesion, migration, and differentiation. Initially, in the nasal mucosa, it has been described in the case of fibrous

mature nasal polyps (Nakagawa, 1999). In the case of allergic rhinitis, fibronectin findings vary according to the stage of the inflammation. In an early stage of allergic rhinitis fibronectin level remains low, in the late stages fibrosis of the mucosal tissue occurs and level of fibronectin- containing structures rises (Hirshoren, 2013). In a case of allergic rhinitis and nasal polyps still can be changed in fibronectin level in nasal mucosa (Van Crombruggen, 2013). Fibronectin could be related to the ongoing question of physicians about an uneffective treatment of allergic rhinitis with topical steroids. Pujolis (2010) described the different reaction to the topical steroids from healthy epithelial cell culture and cell culture from allergic, steroid resistant epithelial cell. The second one increased not only fibronectin production as an answer to the steroid application, but the release of interleukins and cytokines as well. We found increased amount of the fibronectin-containing fibers in the patients nasal and nasopharyngeal mucosa. This fact confirms remodelation of nasal and nasopharyngeal mucosa tissue as a part of isolated postnasal drip pathogenesis.

Interleukin 6 (Il-6) is an inflammatory cytokine responsible for the migration of mast cells and activation of the immune system in a case of infection (Sache, 2010). It is also known that Il-6 can induce a tissue remodelation in the event of inflammation. Il-6 in nasal mucosa is associated with nasal polyp formation (Danielsen, 2006). Il-6 increases in case of reactive/ not-allergic rhinopathy like ozone or air pollution induced rhinopathy (Gronberg, 2003; Ong, 2016). Discussion about Il-6 brings us back to gastroesophageal reflux disease. There still are discussions regarding gastroesophageal reflux and rhinosinusitis, and the leading role in this interaction could play pepsin. In Southwood (2015) study, a correlation between pepsin and Il-6 level in sinus lavage was found. In an everyday clinical praxis detection of pepsin in the nasal fluid is complicated and expensive. In our study gastroesophageal reflux was excluded both, the study and the control group and there was no improvement to the common therapy of proton pump

inhibitors for the patients. However, it's less likely that the pepsin is the reason for Il-6 elevation in the nasal and nasopharyngeal mucosa of patients, further researches are required to establish interaction between pepsin level and Il-6 distribution in the airway tissue not only in some epithelial cells in the sinus lavage. Il-6 finding in our study confirms activation of inflammatory response in nasal and nasopharyngeal mucosa of isolated postnasal drip syndrome patients.

Interleukin 10 (Il-10) is an antiinflammatory cytokine. In allergic rhinitis, it blocks migration of mast cells and eosinophils, realizes the autoregulation of inflammation (Wang, 2014). The level of Il-10 in the tissue samples of nasal polyps remains small, so it confirms Il-10 antagonism to Il-6 (Chen, 2005). Il-10 regulates inflammatory response also in infection. A study describes Il-10 level in nasal tissue in the case of human papillomavirus stays low for not-infected tissue but rises in human papilloma virus infection (Rizzo, 2014). Our study shows increased amount of Il-10 containing structures in the nasal and nasopharyngeal mucosa of isolated postnasal drip patients. This fact together with Il-6 findings confirms balance of inflammatory and antiinflammatory response in isolates postnasal drip syndrome.

Nuclear factor kappa beta (NFkB) in the nasal mucosa is involved in many conditions. In allergic rhinitis, NFkB level correlates with the mucus discharges and the severity of the disease (Wang, 2013). In not-allergic rhinitis, the level of NFkB shows no changes but decreases in case of bacterial infection (Krone, 2013). Valera (2012) describes the interaction between NFkB level and regrowth of nasal polyps, prooving than decreased level of NFkB slows down nasal polyp growing. NFkB increases mucus discharges in a case of airway inflammation by increasing mucus production in goblet cells (Jono, 2002). Sudhoff (2015) according to this fact studies possibility to treat chronic airway diseases and to improve the prognosis of them by decreasing the level of NFkB in airway epithelial cells. Long (2015) published similar study abut NFkB and

allergic rhinitis. Patients with isolated postnasal drip syndrome revealed statistically significant increase of NFkB in the nasal and nasopharyngeal mucosa claiming feasible involving of NFkB in the pathogenesis of this syndrome by increasing of mucus production.

Tumor necrosis factor α (TNFα) is an inflammatory cytokine with various local and systemic effects. TNF α locally is responsible for morphological changes of mucosa like epithelial hyperplasia, vasodilatation and increased vascular permeability, edema of lamina propria and increased production of MUC5CA gene (Kim, 2011). Nasal polyp formation is associated with decreased level of TNFα (Park, 2013). In chronic rhinitis with nasal polyps, the level of TNFα in the tissue doesn't correlate with the TNFα level in the nasal fluid (Oyer 2013). Anfuso (2015) published study confirming a higher level of TNFα in nasal and sinus mucosa in a case of allergic rhinitis with concomitant asthma than allergic rhinitis per se, pointing out the significance of TNFα in the systemic disease more than in local. The increase of TNFα in the bloodstream in a case of chronic sinusitis described Cho (2014). TNF α activates an inflammatory response in the case of *Staphylococcus aureus* infection (Kohanski, 2015). In the event of pollution-induced rhinitis, TNFα level dropped below the control's TNFα level in the nasal fluid (Hellgran, 2009). In our study, the patients nasal and nasopharyngeal mucosa contained just a few TNFα immunopositive structures, however, isolated postnasal drip syndrome patients got more TNFα immunopositive structures by compering with the controls. This fact suggests about the local involving of TNFα in the pathogenesis of isolated postnasal drip syndrome and explains the lac of systemic ones because of the low level of this cytokine.

Beta defensing is a natural antimicrobial protein of airway mucosa. It is a major component of the innative immune system. Increased level of B defensing is described in the virus and bacterial infection (Lillard, 1999; Proud, 2004). In the case of nasal polyps, the evidence about the expression of

B defensine is controversial. There are publications claiming to increase of B defensing in polyp tissue (Pacova, 2004) and denying it (Chen, 2004). Decreased level of B defensin in the nasal tissue is associated with intracellular bacterial invasion (Zanger, 2011). However, Laudien (2011) described the various level of B defending in nasal tissue of Wegener's granulomatosis patients and local colonization with Staphylococcus aureus did not correlate with decreased level of B defensin. Our study reveals increased level of B defensing in nasal and nasopharyngeal mucosa of the patients. This finding suggests about activation of the local immune system in the case of isolated postnasal drip syndrome.

Overall, our study is urgent and innovative. Up to now, there is no similar study describing isolated postnasal drip syndrome in the clinical and morphological aspect of mucosal inflammation. Our data claim about chronic unspecific inflammation is in the routine histological examination. There is an activation of the innate immune system and proinflammatory and antiinflammatory cytokines, induction of apoptosis and tissue remodelation in the isolated postnasal drip syndrome.

The study is very relevant in an aspect of the number of involved people. Because of legal reasons, studies are done on animals or cell cultures. Examinations of nasal and nasopharyngeal tissue species from 40 people, visualizing structures of mucosa and identifying the localization of immunopositive structures are of very high importance for building up proceses in current condition.

4. CONCLUSIONS

1. Patients with the isolated postnasal drip syndrome have a chronic inflammation with lymphocyte infiltration, thickened basal membrane and hyperplasia of submucosal glands.
2. Increased number of PGP 9.5-containing structures in the nasal and nasopharyngeal mucosa of isolated post nasal syndrome patients confirms increased neuropeptide innervation including sympathetic (NPY containing), but sensory (CGRP, SP containing) nerve fibers.
3. Increased number of VIP-containing nerve fibers suggests about activation of the parasympathetic nervous system, resulting as a clinical symptom of mucus discharges in the nasopharynx.
4. Increased number of chromogranin A-containing structures (cells and fibers) proves activation of the neuroendocrine system in the nasal, and especially in the nasopharyngeal mucosa of isolated postnasal syndrome patients.
5. MMP-9 and fibronectin immunopositive structures in the patient's *lamina propria* point out tissue remodelation process in the isolated postnasal drip syndrome.
6. Statistically significantly increased the number of caspases-containing cells in the patient's nasal and nasopharyngeal mucosa proves apoptosis activation in isolated postnasal drip syndrome.
7. The increased finding of proinflammatory (Il-6) and anti-inflammatory (Il-10) cytokines suggest about relative balance between inflammation/anti-inflammation process in the nasal and nasopharyngeal mucosa of isolated postnasal drip patients.
8. Increased number of β defensine and NFkB containing structures in the nasal and nasopharyngeal mucosa of isolated postnasal drip syndrome explains local hypersecretion of submucosal glands in the isolated postnasal drip syndrome.

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LIST OF PUBLICATIONS

Scientific papers (4):

1. Sumeraga G., Pilmane M., Kise L. Neuropeptides in the Nasal and Nasopharyngeal Mucosa in Patients with Postnasal Drip Syndrome. Riga Stradins University Collection of Scientific Papers, 2011. 93–100.
2. Sumeraga G., Pilmane M. Distribution of neuropeptides in nasal and nasopharyngeal mucosa in patients with the postnasal drip syndrome. Papers on Anthropology XX, 2011, 389–404.
3. Sumeraga G., Pilmane M., Kise L. Tissue remodeling process in upper airway mucosa in case of isolated postnasal drip syndrome. Theory and Practice in Medicine. 2012; 18(vol.3):260–266.
4. Sumeraga G., Pimane M., Kise L. Распределение интерлейкинов и цитокинов в слизистой оболочке носа и носоглотки при изолированном синдроме постназального затекания. Вестник оториноларингологии. Accepted for the publication 2017.

Abstracts and papers in the international conferences (6):

1. Sumeraga G., Pilmane M., Kise L. The distribution and appearance of neuropeptides in the nasal and nasopharyngeal mucosa of patients with post nasal drip syndrome.1st Congress of CE-ORL-HNS, Barcelona, Spain 2011.02.–06.07. (Oral presentation).
2. Sumeraga G., Pilmane M., Kise L. The distribution of neuropeptides in nasal and nasopharyngeal mucosa in patients with post nasal drip syndrome. 5th Baltic Otorhinolaryngology congress. Riga, Latvia, 2011. 16.–18.09. (Oral presentation).
3. Sumeraga G., Pilmane M. Distribution of neuropeptides in nasal and nasopharyngeal mucosa in patients with post nasal drip syndrome. Baltic

Morphology VI, Tartu, Estonia. 2011.22.–23.09. (Stenda referāts). Sumeraga G., Pilmane M., Kise L. The implication of neurogenic inflammation and tissue remodeling process in upper airway mucosa on pathogenesis of isolated postnasal drip syndrome. 24. Congress of the European Rhinologic Society, Touluse, France, 2012. FP-85; Thesis. (Oral presentation).

4. Baltic Morphology VII Conference. The role of cytokines and neurogenic inflammation in case of isolated postnasal drip syndrome. 2013.08.11. Rīga, Latvia (Oral presentation).
5. 2nd Meeting of European Academy of ORL-HNS and CE ORL-HNS. Identification of apoptosis in the nasal and nasopharyngeal mucosa in case of isolated postnasal drip syndrome. 2013.27.04.–01.05. Nica, Francija (Oral presentation).

Abstracts and thesis in the local conferences in Latvia (6):

1. Sumeraga G., Ķīse L., Pilmane M. Neiropeptīdu sadalījums deguna un aizdegunes gļotādā pacientiem ar aizdegunes tecēšanas sindromu. Rīgas Stradiņa universitātes 2011. gada Zinātniskās konferences tēzes. 86. lpp. (Oral presentation).
2. Sumeraga G., Pilmane M., Ķīse L. Augšējo elpceļu gļotādas audu remodelācija pacientiem ar aizdegunes tecēšanas sindromu. Rīgas Stradiņa universitātes 2012. gada Zinātniskās konferences tēzes. 82. lpp. (Oral presentation).
3. Sumeraga G., Pilmane M., Ķīse L. Deguna un aizdegunes gļotādas audu apoptozes noteikšana pacientiem ar aizdegunes tecēšanas sindromu. RSU 2013. gada Zinātniskās konferences tēzes. 137. lpp. (Poster presentation).
4. Sumeraga G., Pilmane M. Izolēts aizdegunes tecēšanas sindroms – deguna un aizdegunes gļotādas audu morfoloģiskais un imūnhistoķīmiskais

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5. Sumeraga G., Pilmane M. Deguna gļotādas morfoloģiskā un imūnhistoķīmiskā analīze pacientiem ar klīniski nozīmīgu deguna starpsienas deviāciju. 2015. gada RSU Zinātniskās konferences tēzes. 82. lpp. (Oral presentation).
6. Sumeraga G., Pilmane M. Deguna gļotādas morfoloģiskā un imūnhistoķīmiskā analīze izolēta aizdegunes tecēšanas sindroma pacientiem. 2016. gada RSU Zinātniskās konferences tēzes. 93. lpp. (Poster presentation).