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Expressions of MMP2, MMP9, and TIMP-1 in the inflammatory cells of nasal polyps: granulocytes, monocytes, and mast cells

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Abstract. – OBJECTIVE: We investigated the role of matrix metalloproteinase-2 (MMP-2) and MMP-9 in nasal polyp (NP) pathogenesis.

PATIENTS AND METHODS: In group 1 (n = 24), polyp specimens were obtained from maxillary sinus, ethmoid sinus, and nasal cavity. In group 2 without NP (control) (n = 11), inferior turbinate samples were taken. Inflammatory cell count and MMP2, MMP9 and tissue inhibitor of metalloproteinase-1 (TIMP-1), positivity indexes (PIs) were evaluated.

RESULTS: Granulocyte and mast cell-MMP2 and MMP9-PI were higher than the rate of monocyte-MMP2-PI and monocyte-MMP9-PI, respectively, in the ethmoid sinus, maxillary sinus, and nasal cavity. Mast Cell-TIMP1-PI was higher than the rates of granulocyte-TIMP1-PI and monocyte-TIMP1-PI in the maxillary sinus and was higher than the rate of monocyte-TIMP1-PI in the ethmoid sinus.

CONCLUSIONS: Excessive MMP2 and MMP9, compared to TIMP1, are present in granulocytes and mast cells, respectively. With matrix MMPs, the extracellular matrix is destroyed, leading inflammatory cells to pass through, causing polypoid degeneration.

Key Words:
Matrix metalloproteinase-2 (MMP-2), Matrix metalloproteinase-9 (MMP-9), Tissue inhibitor of metalloproteinase-1 (TIMP-1), Inflammatory cells, Nasal polyp.

Introduction

Nasal polyps appear as grape-like structures in the nasal cavity. The stroma of mature polyps is mainly characterized by its edematous nature and consists of supporting fibroblasts and infiltrating inflammatory cells, localized around "empty" pseudo cyst formations[1,2]. The common epithelial type in the polyp tissue is pseudo stratified ciliated cylindric epithelium, which contains goblet cells. Moreover, mast cells and eosinophils may have a role in the inflammatory processes, leading to nasal polyposis formation[3,4].

Matrix metalloproteinases (MMPs) are a subfamily of zinc- and calcium-dependent enzymes belonging to the metzincin superfamily. The two gelatinases (MMP-2 and -9) contain three FnII-like repeats in their catalytic domain. Most of the MMPs are secreted as proenzymes and their activation occurs in the pericellular and extracellular space. Various MMPs are localized also inside the cells. This may partly explain the ability of some MMPs to process intracellular proteins and further demonstrates the complex roles of MMPs under physiological and pathological conditions[5-7].

Di Lorenzo et al[8] reported that chronic eosinophil mucosal inflammatory disease in NP involves a self-sustaining mechanism, i.e., local release of inflammatory mediators, independent of allergen stimulation of nasal mucosa. Increased release of inflammatory mediators contributes to the development of nasal polyps, determining edema and an increased recruitment of inflammatory cells. As well as eosinophils, mast cells also play a key role in this process. Takasaka et al[9] reported neutrophils (PMN) migration and attachment to the basal lamina and accelerated degranulation of mast cells.

The question remains as to why "ballooning" of mucosa develops in polyposis patients and not in all rhinosinusitis patients. Nasal polyps have a

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strong tendency to recur after surgery even when aeration is improved[10]. In the present study, we investigated the inflammatory cells of nasal polyps: granulocytes, monocytes, and mast cells. We also investigated the distribution of MMP2, MMP9 and TIMP-1 in the inflammatory cells of the nasal polyps to evaluate the pathogenesis.

Patients and Methods

This study was conducted at the Ear Nose Throat (ENT) Department of Kirikkale University Medical School. The immunohistochemical staining and light microscopic examination were performed at the Pathology Department of Kirikkale University Medical School. All the stages of the study were planned and conducted with the approval of the Kirikkale University Faculty of Medicine Local Ethics Committee (Date: 23.03.2009, No.: 2009/028) according to the principles outlined in the Declaration of Helsinki. This study was supported by the funds provided by Kirikkale University Scientific Research Projects Unit (2009/16).

Subjects

The nasal polyp group was comprised of the patients examined in the Otolaryngology Department of Kirikkale University Medical School. They used topical corticosteroid nasal spray for at least 6-week duration, and if the pathology persisted, an operation was performed. This study group consisted of 24 adult patients (21 male, 3 female) with nasal polyps, who underwent Functional Endoscopic Sinus Surgery (FESS). The mean age of the patients was 45.97 ± 11.60 years (range: 23-70 years).

The control group consisted of 11 adult patients without nasal polyps (6 male and 5 female), who underwent septoplasty operation. The mean age of the control subjects was 29.90 ± 14.22 years (range: 18-56 years). No accompanying diseases were determined in the patients or the subjects[11-15].

For the study and control group, retrospective data (data and previously prepared slides)[11-15] were used in the other studies of the authors which were published[11-15]. The difference of this study was to evaluate the distribution of MMP2, MMP9 and TIMP-1 in the inflammatory cells of the nasal polyps and the retrospective data related to these topics were used.

Methods

Questionnaire

Anterior and posterior nasal discharge, nasal congestion, cough, facial and dental pain, halitosis, paroxysmal nocturnal coughing spells, sore throat, fever, olfactory loss, headache and ear pain knowledge were evaluated[11-16].

Endoscopic examination

Endoscopic examination with 0º and 30º endoscopes were performed in the Endoscopy Unit of ENT Department of Kirikkale University Medical School. Discharge (none, clear and thin, thick, purulent), mucosal status (normoplasia, light hyperplasia with no erythema, hyperplasia)[17], anatomic abnormalities (septal deviation, lateral rotation of the uncinate process, turbinate hypertrophy and other anatomic abnormalities)[16], and localization and size of the polyps were examined[11-15].

In preoperative nasal endoscopic examination of the study group, appearances of nasal polyps were staged based on Lawson's criteria (1991)[18] as Stage 0: No polyp presented, Stage 1: Polyp under the medial turbinate that was detected by endoscopy, Stage 2: Protruding polyp in the medial turbinate that was detected without using endoscopy, Stage 3: Massive polyposis.

Computed tomography

By axial and coronal sections of the paranasal sinuses, in the nasal polyp group, locations and sizes of the polyps in the nasal cavity and paranasal sinuses were evaluated. In addition, pan-polyposis, septal deviation, concha bullosa, lateral rotation of the uncinate process, prominent ethmoid bulla, and other anatomic abnormalities[16] were also investigated. In the control group, evaluations were made using both coronal and axial computed tomography images[11-15].

Operation

All the patients in the study group underwent FESS for nasal polyposis. Biopsies were performed under general anesthesia. Samples were obtained from macroscopically observed polypoid areas. Specimens including polyp tissue were excised from 3 regions: nasal cavity, maxillary and ethmoid (anterior and posterior) sinuses. The specimens were examined at x400 magnification under light microscopy, and only the slides with polypoid tissue were included in the study. The tissues, which were edematous and rich in vessels; had severe inflammatory cells and showed polypoid development, were included in the study

as the polyp group. Slides including chronic inflammatory process without polypoid tissue were excluded from the study. Finally, the study group consisted of the specimens from three regions: the ethmoid sinuses (including 16 specimens), the maxillary sinus (including 10 specimens), and the nasal cavity (including 10 specimens).

In the control group, specimens were collected *via* punch biopsies from the inferior turbinates during septoplasty operation, and 11 specimens were included in the control group[11-15].

Immunohistochemical staining

In the study and control groups, surgical specimens were examined by immunohistochemical staining technique with monoclonal antibodies against MMP-2, MMP-9 and TIMP-1. In each of the surgical specimens, the rate of MMP-2, MMP-9 and TIMP-1 positivity were evaluated at a 3-4 high magnification field under light microscope and inflammatory cell count [Mast cells, granulocytes and other mononuclear cells (lymphocytes and plasma cells)] were assessed by counting a total of 100 cells consisting of inflammatory cells at 3-4 high magnification field, and the means were calculated. Eventually, in each inflammatory cell group (mast cells, granulocytes and other mononuclear cells), the means of the MMP2+ cells, MMP9+ cells, and TIMP-1 (+) cells per 100 cells at a high magnification field (x400) were detected.

Immunohistochemical Staining Technique

Sections of 5 μ thickness were obtained, transferred to adhesive slides, and dried in autoclave at 37°C overnight and at 60°C for 20 minutes. They were deparaffinized and dehydrated by immersion into xylene, twice for ten minutes and in alcohol, twice for ten minutes. The specimen was then incubated in 3% H_2O_2 for five minutes to inhibit endogenous peroxidases. The preparations were transferred into citrate-based antigen retrieval solution (Dako; Glostrup, Denmark; pH: 6) for MMP2, MMP9 and TIMP-1 antigens (Lab Vision Corporation Neomarkers, Fremont, CA, USA). All slides were kept in microwave oven (750 watts) twice for five minutes. By using Shandon Sequeza Tm (Sequenza, Shandon, Amsterdam, The Netherlands), manual staining device for standardization, classical Streptavidine Avidin-Biotin-Peroxidase (Strept. AB-Peroxidase) method and diaminobenzidine (DAB) chromogen (20 minutes) were applied for immunohistochemical analysis of three antibodies. Non-immune mouse serum served as a negative control and Mayer's haematoxylin was used as counterstain. Cytoplasmic staining was considered evidence of positivity.

The slides were reviewed by an expert pathologist. In each slide, the number of MMP2+, MMP9+ and TIMP-1+ inflammatory cells [Mast cells, granulocytes and other mononuclear cells (lymphocytes and plasma cells)] were counted under a light microscope (Leica, Wetzlar, Mannheim, Germany) per 100 cells at 3-4 high magnification field. The means of the cell counts were calculated. MMP-PI for MMP-2, MMP-9 and TIMP-1 were detected at a scale of 0-3 in a SE and deep layers of the lamina propria.

Positivity index (PI)

For the quantitative assessment of MMP2, MMP9 and TIMP-1 expressions, staining in the inflammatory cells (mast cells, granulocytes and other mononuclear cells) were assessed by counting a total of 100 cells consisting of inflammatory cells at a 3-4 high magnification field and the means were calculated. Eventually the means of the MMP2+ cells, MMP9+ cells, and TIMP-1+ cells per 100 cells at a high magnification field (x400) were detected. Scoring was performed on a 0-3 scale, where 0 represented negative staining; 1, weakly positive; 2, positive; and 3, strongly positive[19]:

PI 0 means that antigen (MMP2, MMP9, and TIMP-1) + cell count was 0% (no stained cells);
PI 1 means that antigen (MMP2, MMP9, and TIMP-1) + cell count was < 5%;
PI 2 means that antigen (MMP2, MMP9, and TIMP-1) + cell count was 5-50%;
PI 3 means that antigen (MMP2, MMP9, and TIMP-1) + cell count was > 50%;

The inflammatory cells (mast cells, granulocytes and other mononuclear cells) of the lamina propria were evaluated.

Statistical Analysis

The data obtained in the study were analyzed using SPSS for Windows 16.0 software (SPSS Inc., Chicago, IL, USA). For evaluation of the differences among the four groups (ethmoid sinus, maxillary sinus, nasal cavity and control) Kruskal-Wallis variance analysis was used. Kruskal-Wallis variance analysis was used to determine the differences between inflammatory cell counts (%); MMP2; MMP9; and TIMP-1 positivity of inflammatory cells. When a statistically sig-

nificant result was found, pair-wise comparisons were performed to find the value which caused the difference by Wilcoxon Signed Ranks Test with Bonferroni Correction. To analyze the correlations, Pearson's Correlation Test or Spearman's Correlation Rho Efficient test were used. $p < 0.05$ was considered statistically significant.

Results

Inflammatory cell count (%), MMP2, MMP9 and TIMP-1 positivity index levels of the inflammatory cells (Figures 1-4) in the ethmoid sinus, maxillary sinus, nasal cavity and control groups were shown as mean ± standard deviation and minimum and maximum in Table I.

The differences between all four groups (ethmoid sinus, maxillary sinus, nasal cavity and control) for inflammatory cell count (%) (granulocytes, monocytes and mast cells) and for MMP2 positivity of inflammatory cells (granulocyte-MMP2-PI, monocyte-MMP2-PI, mast cell-MMP2-PI), MMP9 positivity of inflammatory cells (granulocyte-MMP9-PI, monocyte-MMP9-PI, mast cell-MMP9-PI) and TIMP-1 positivity of inflammatory cells (granulocyte-TIMP-1-PI, monocyte-TIMP-1-PI, mast cell-TIMP-1-PI) separately were analyzed by Kruskal-Wallis' variance analysis (Table I):

- For inflammatory cell count (%), statistically significant difference was found in the granulocytes ($p = 0.000$).
- For MMP2 positivity, the difference was statistically significant in granulocyte-MMP2-PI ($p = 0.002$); and mast cell-MMP2-PI ($p = 0.003$).
- For MMP9 positivity, the difference was statistically significant in granulocyte-MMP2-PI ($p = 0.000$); and mast cell-MMP2-PI ($p = 0.001$) (Table I).

To find the value which caused the difference, pair-wise comparisons were performed by Wilcoxon Signed Ranks Test with Bonferroni Correction (Table II).

- For inflammatory cell count (%), granulocyte values of the ethmoid sinus ($p = 0.003$, z = -2.940) and maxillary sinus ($p = 0.012$, z = -2.524) were significantly higher than the granulocyte value of the control group.
- For MMP2 positivity, granulocyte-MMP2-PI values of the ethmoid sinus ($p = 0.010$, z = -2.565) and nasal cavity ($p = 0.011$, z = -2.537) were significantly higher than that of the control group.

Mast cell-MMP2-PI value of the nasal cavity ($p = 0.011$, z = -2.549) was significantly higher than that of the control group.

- For MMP9 positivity, granulocytes-MMP9-PI values of the ethmoid sinus ($p = 0.004$, z = -2.913) and nasal cavity ($p = 0.007$, z = 2.701) were significantly higher than that of the control group.

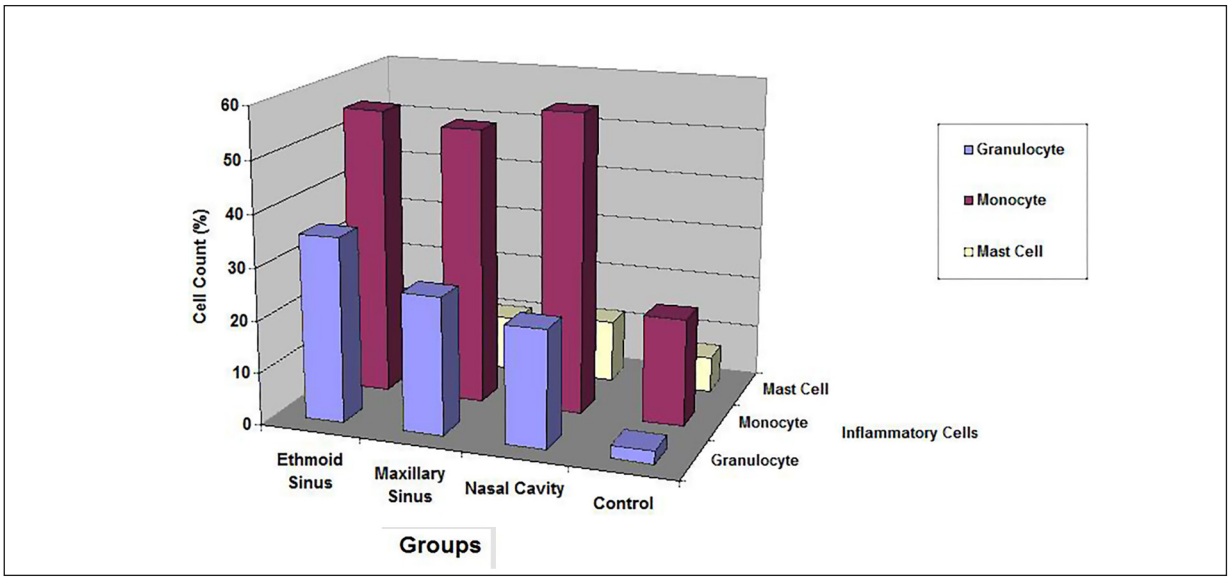

Figure 1. Inflammatory cell count (%) in the ethmoid sinus, maxillary sinus, nasal cavity and control groups.

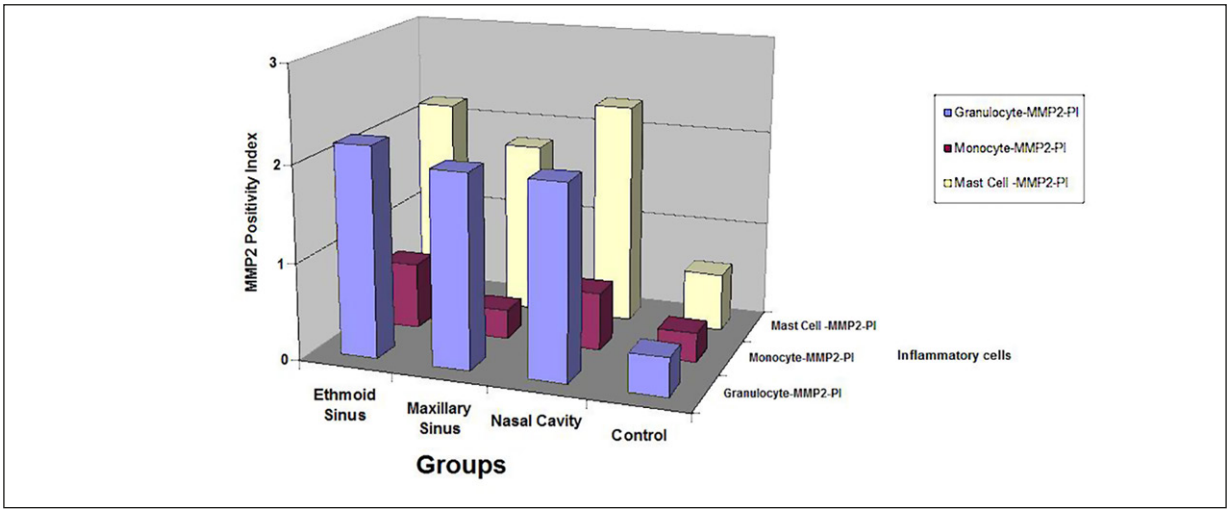

Figure 2. MMP2 positivity index levels in the inflammatory cells of the ethmoid sinus, maxillary sinus, nasal cavity and control groups.

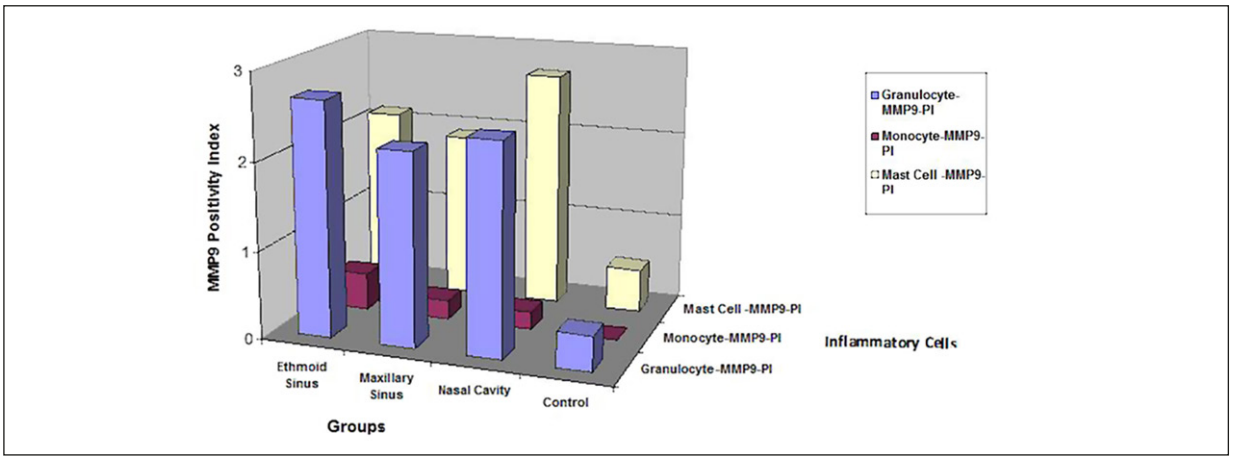

Figure 3. MMP9 positivity index levels in the inflammatory cells of the ethmoid sinus, maxillary sinus, nasal cavity and control groups.

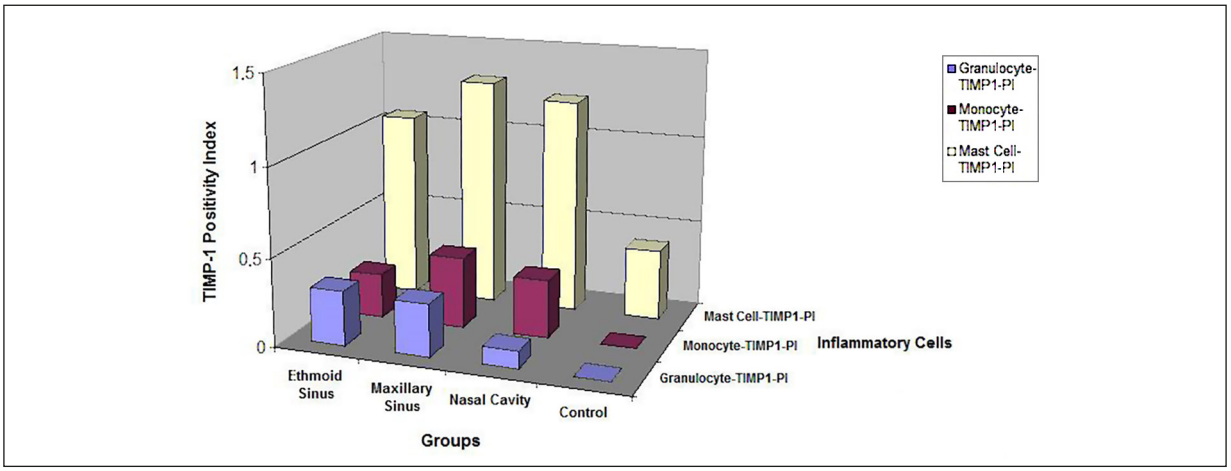

Figure 4. TIMP-1 positivity index levels in the inflammatory cells of the ethmoid sinus, maxillary sinus, nasal cavity and control groups.

Table I. MMP2, MMP9 and TIMP-1 positivity index levels of the inflammatory cells in the ethmoid sinus, maxillary sinus, nasal cavity and control groups.

	Groups																
	Control				Nasal cavity				Maxillary sinus				Ethmoid sinus				*p**
	Mean	Std. Dev.	Min	Max	Mean	Std. Dev.	Min	Max	Mean	Std. Dev.	Min	Max	Mean	Std. Dev.	Min	Max	
Inflammatory cell count (%)**																	
Granulocytes	2.50	5.40	0.00	15.00	22.50	14.19	0.00	40.00	26.40	22.72	0.00	60.00	35.62	21.89	5.00	75.00	0.000
Monocytes	20.50	33.20	0.00	75.00	57.50	24.18	0.00	80.00	53.00	28.59	0.00	95.00	55.31	19.27	25.00	90.00	0.058
Mast cell	7.00	13.37	0.00	40.00	12.00	6.32	5.00	20.00	10.60	8.18	0.00	30.00	9.06	8.20	0.00	35.00	0.069
*p**	0.700				0.001				0.004				0.000				
MMP2																	
Granulocyte-MMP2-PI	0.40	0.96	0.00	3.00	2.00	0.81	0.00	3.00	2.00	1.05	0.00	3.00	2.18	0.75	1.00	3.00	0.002
Monocyte-MMP2-PI	0.30	0.67	0.00	2.00	0.60	0.69	0.00	2.00	0.30	0.48	0.00	1.00	0.68	0.79	0.00	2.00	0.370
Mast Cell-MMP2-PI	0.60	0.96	0.00	2.00	2.30	0.94	0.00	3.00	1.80	1.03	0.00	3.00	2.18	0.98	0.00	3.00	0.003
*p**	0.797				0.001				0.000				0.000				
MMP9																	
Granulocyte-MMP9-PI	0.40	0.84	0.00	2.00	2.40	0.96	0.00	3.00	2.20	0.91	0.00	3.00	2.68	0.47	2.00	3.00	0.000
Monocyte-MMP9-PI	0.00	0.00	0.00	0.00	0.20	0.42	0.00	1.00	0.22	0.44	0.00	1.00	0.43	0.62	0.00	2.00	0.166
Mast cell-MMP9-PI	0.50	1.08	0.00	3.00	2.70	0.48	2.00	3.00	1.90	0.99	0.00	3.00	2.12	0.88	0.00	3.00	0.001
*p**	0.332				0.000				0.000				0.000				
TIMP-1																	
Granulocyte-TIMP-1-PI	0.00	0.00	0.00	0.00	0.10	0.31	0.00	1.00	0.30	0.48	0.00	1.00	0.31	0.47	0.00	1.00	0.173
Monocyte-TIMP-1-PI	0.00	0.00	0.00	0.00	0.33	0.50	0.00	1.00	0.40	0.51	0.00	1.00	0.25	0.44	0.00	1.00	0.185
Mast cell-TIMP-1-PI	0.40	0.96	0.00	3.00	1.22	0.97	0.00	2.00	1.30	0.82	0.00	3.00	1.06	1.12	0.00	3.00	0.101
*p**	0.127				0.023				0.006				0.040				

**p*-value shows the results of Kruskal-Wallis' variance analysis. **Inflammatory cell counts were assessed by counting a total of 100 cells consisting of mainly inflammatory cells at 3-4 high magnification field and the means were calculated.

Table II. Pairwise comparisons by Wilcoxon Signed Ranks Test with Bonferroni Correction*.

	Ethmoid sinus-maxillary sinus		Ethmoid sinus-nasal cavity		Ethmoid sinus-control		Maxillary inus-nasal cavity		Maxillary sinus-control		Nasa cavity-control	
	z	p	z	p	z	p	z	p	z	p	z	p
Granulocytes	-1.785	0.074	-1.278	0.201	-2.940	0.003	-0.356	0.722	-2.524	0.012	-2.388	0.017
Granulocyte-MMP2-PI	-0.750	0.453	-1.342	0.180	-2.565	0.010	-0.106	0.915	-2.013	0.044	-2.537	0.011
Mast cell-MMP2-PI	-0.877	0.380	-0.138	0.890	-2.360	0.018	-0.863	0.388	2.264	0.024	-2.549	0.011
Granulocyte-MMP9-PI	-1.633	0.102	-0.707	0.480	-2.913	0.004	-0.702	0.483	-2.428	0.015	2.701	0.007
Mast cell-MMP9-PI	-0.816	0.414	-1.265	0.206	-2.598	0.009	-1.994	0.046	-2.392	0.017	-2.724	0.006

*$p < 0.0125$ was statistically significant.

Mast cell-MMP9-PI values of the ethmoid sinus (p = 0.009, z = -2.598) and nasal cavity (p = 0.006, z = 2.724) were significantly higher than that of the control group (Table II).

For each of the four groups (ethmoid sinus, maxillary sinus, nasal cavity and control) separately, the differences between the inflammatory cell count (%) (granulocytes, monocytes, mast cells), MMP2 positivity of inflammatory cells (granulocyte-MMP2-PI, monocyte-MMP2-PI, mast cell-MMP2-PI); MMP9 positivity of inflammatory cells (granulocyte-MMP9-PI, monocyte-MMP9-PI, mast cell-MMP9-PI); and TIMP-1 positivity of inflammatory cells (granulocyte-TIMP1-PI, monocyte-TIMP1-PI, mast cell-TIMP1-PI) were analyzed by Kruskal-Wallis variance analysis (Table I).

In the ethmoid sinus, maxillary sinus, and nasal cavity, the differences between the inflammatory cell count (%) (p = 0.001, p = 0.004 and p = 0.000 respectively); MMP2 positivity (p = 0.001, p = 0.000 and p = 0.000 respectively), and MMP9 positivity (p = 0.000, p = 0.000 and p = 0.000 respectively) were statistically significant (Table I).

To determine the value which caused the difference, pairwise comparisons were performed by Wilcoxon Signed Ranks Test with Bonferroni Correction (Table III):

For inflammatory cell count (%):

- In the ethmoid sinus, the granulocyte count (p = 0.003, z = -2.932) and monocyte count (p = 0.000, z = -3.523) were significantly higher than the mast cell count.
- In the maxillary sinus, the monocyte count was significantly higher than the mast cell count (p = 0.007, z = -2.677).
- In the nasal cavity, the monocyte count was significantly higher than the granulocyte count (p = 0.007, z = -2.677); and mast cells (p = 0.007, z = -2.712) (Table III).

For MMP2 positivity:

- The rate of granulocyte-MMP2-PI was significantly higher than that of monocyte-MMP2-PI in the ethmoid sinus (p = 0.001, z = -3.384), maxillary sinus (p = 0.007, z = 2.701) and nasal cavity (p = 0.008, z = -2.640)
- The rate of mast cell-MMP2-PI was significantly higher than that of monocyte-MMP2-PI in the ethmoid sinus (p = 0.002, z = -3.101), maxillary sinus (p = 0.011, z = -2.549) and nasal cavity (p = 0.007, z = -2.714) (Table III).

For MMP9 positivity:

- The rate of granulocyte-MMP9-PI was significantly higher than that of monocyte-MMP9-PI in the ethmoid sinus (p = 0.000, z = -3.630), maxillary sinus (p = 0.010, z = -2.588) and nasal cavity (p = 0.006, z = -2.739)
- The rate of mast cell-MMP9-PI was significantly higher than that of monocyte-MMP9-PI in the ethmoid sinus (p = 0.001, z = -3.354),

Table III. Pairwise comparisons by Wilcoxon Signed Ranks Test with Bonferroni Correction*.

	Nasal cavity		Maxillary sinus		Ethmoid sinus	
	z	p	z	p	z	p
Inflammatory cell count (%)**						
Granulocyte - Monocyte	-2.677	0.007	-1.849	0.064	-1.890	0.059
Granulocyte -Mast cell	-1.846	0.065	-1.897	0.058	-2.932	0.003
Monocyte -Mast cell	-2.712	0.007	-2.677	0.007	-3.523	0.000
MMP2						
Granulocyte-MMP2-PI and Monocyte -MMP2-PI	-2.640	0.008	-2.701	0.007	-3.384	0.001
Granulocyte-MMP2-PI and Mast Cell -MMP2-PI	-1.732	0.083	-0.707	0.480	-0.137	0.891
Monocyte -MMP2-PI and Mast Cell -MMP2-PI	-2.714	0.007	-2.549	0.011	-3.101	0.002
MMP9						
Granulocyte-MMP9-PI and Monocyte–MMP9-PI	-2.739	0.006	-2.588	0.010	-3.630	0.000
Granulocyte-MMP9-PI and Mast Cell –MMP9-PI	-1.342	0.180	-1.342	0.180	-2.309	0.021
Monocyte–MMP9-PI and Mast Cell –MMP9-PI	-2.879	0.004	-2.588	0.010	-3.354	0.001
TIMP1						
Granulocyte-TIMP1-PI and Monocyte –TIMP1-PI	-1.414	0.157	-1.000	0.317	-0.378	0.705
Granulocyte-TIMP1-PI and Mast Cell –TIMP1-PI	-2.271	0.023	-2.456	0.014	-2.220	0.026
Monocyte –TIMP1-PI and Mast Cell –TIMP1-PI	-2.070	0.038	-2.460	0.014	-2.565	0.010

*p < 0.0175 was statistically significant. **Inflammatory cell counts were assessed by counting a total of 100 cells consisting of mainly inflammatory cells at 3-4 high magnification field and the means were calculated.

maxillary sinus ($p = 0.010$, z = -2.588) and nasal cavity ($p = 0.004$, z = -2.879) (Table III).

For TIMP-1 positivity:

- In the maxillary sinus, the rate of mast cell-TIMP-1-PI was significantly higher than that of granulocyte-TIMP-1-PI ($p = 0.014$, z = -2.456).
- The rate of mast cell-TIMP-1-PI was significantly higher than that of monocyte-TIMP-1-PI in the ethmoid sinus ($p = 0.010$, z = -2.565) and maxillary sinus ($p = 0.014$, z = -2.460) (Table III).

In the study group, correlations between age, polyp duration, Brinkman Index and each of the inflammatory cell count and MMP2 positivity index levels of the inflammatory cells (Table IV) and MMP9 and TIMP1 positivity index levels (Table V) in the ethmoid sinus, maxillary sinus and nasal cavity were analyzed by Pearson Correlation Test. Correlations between gender, smoking status, each of the inflammatory cell count, MMP2 positivity index levels in the inflammatory cells (Table IV), MMP9 and TIMP1 positivity index levels (Table V) in the ethmoid sinus, maxillary sinus, and nasal cavity were analyzed by Spearman Correlation Rho Efficient:

- With increased age, the rate of mast cell-MMP9-PI increased in the ethmoid sinus ($p = 0.040$, r = 0.519), while the rate of monocyte-MMP9-PI decreased in the nasal cavity ($p = 0.040$, r = -0.654).
- In the ethmoid sinus, the rate of monocyte-TIMP-1-PI decreased in males and increased in females ($p = 0.006$, r = -0.655).
- With increased polyp duration, the rate of granulocyte-MMP2-PI increased in the ethmoid sinus ($p = 0.028$, r = 0.630) and decreased in the maxillary sinus ($p = 0.025$, r = -0.816).
- In the patients who smoked, the mast cell count decreased in the maxillary sinus ($p = 0.046$, r = -0.764).

Histopathologic Findings

On light microscopy examination, pseudo stratified ciliated epithelia were present in the majority of the polyps, and a very small ratio of the polyps were also lined with metaplastic epithelium. In all the samples, mononuclear cells (lymphocytes and plasma cells) were predominant compared to other inflammatory cells (granulocytes and mast cells). Inflammatory cells had a tendency to locate around the blood vessels. Stromal edema was also seen.

In the MMP2 and MMP9 stained sections, strong positivity was specifically detected in granulocyte and mast cell cytoplasms. In the mononuclear cells, there was either no or minimally positive staining (Figures 5-6). TIMP-1 expression was significantly lower compared to MMP2 and MMP9 expressions. It was prominent in the mast cell cytoplasms and was minimal in the other inflammatory cells (Figure 7).

Discussion

Nasal polyps consist in loosing connective tissue, edema, inflammatory cells and some glands and capillaries, and are covered with varying types of epithelium, mostly respiratory pseudo stratified epithelium with ciliated cells and goblet cells. Eosinophils, neutrophils, mast cells, plasma cells, lymphocytes and monocytes are also present, as well as fibroblasts, in nasal polyps[1,2].

MMPs are the major proteolytic enzymes involved in extracellular matrix (ECM) damage or repair[20]. MMP-2 and MMP-9 have catalytic site of the enzymes which cause the degradation of macromolecules such as elastin, gelatin and collagens IV, V and XI[21-27]. In nasal polyposis, the presence of MMP-9 positive inflammatory cells around and inside the pseudocyst formation shows their direct role in the degradation of the ECM. Once chemo-attracted, both inflammatory and endothelial cells express MMPs for diapedesis and help the inflammatory cells to migrate inside the ECM. In function of the affected tissue or the underlying disease, different kinds of MMP/TIMP balances can be found: imbalance or co-regulation[20].

In the present study, the rates of granulocyte (ethmoid sinus and maxillary sinus); mast cell-MMP2-PI (nasal cavity); granulocyte-MMP2 and MMP9-PIs, and mast cell-MMP9-PI (ethmoid sinus and nasal cavity) were higher than the control group. The granulocyte, monocyte count (ethmoid sinus) and the monocyte count (maxillary sinus) were higher than the mast cell count. In the nasal cavity, the monocyte count was higher than granulocyte and mast cell counts. Granulocyte and mast cell MMP2 and MMP9-PIs were higher than monocyte-MMP2 and MMP9-PIs (ethmoid sinus, maxillary sinus and nasal cavity). Mast cell-TIMP-1-PI was higher than granulocyte and monocyte TIMP-1-PIs (maxillary sinus); and monocyte-TIMP-1-PI (ethmoid sinus).

As seen in Figures 2, 3 and 4, in the ethmoid sinus, maxillary sinus and nasal cavity polyps, the rates of granulocyte-PI (MMP2, MMP9) and

Table IV. Correlations between age, gender, polyp duration, smoking and Brinkman Index; and inflammatory cell count and MMP2 positivity index levels in the inflammatory cells of the ethmoid sinus, maxillary sinus and nasal cavity.

		Inflammatory cell count (%)						MMP2 positivity index					
		Granulocyte		Monocyte		Mast cell		Granulocyte- MMP9-PI		Monocyte-MMP9-PI		Mast cell-MMP9-PI	
		r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Ethmoid	Age*	0.113	0.676	-0.198	0.463	0.162	0.548	-0.306	0.249	-0.096	0.722	-0.186	0.491
	Gender**	0.351	0.183	-0.432	0.095	-0.022	0.935	-0.442	0.086	-0.112	0.680	-0.404	0.121
	Duration*	-0.045	0.890	0.071	0.826	-0.043	0.894	0.630	0.028	0.506	0.093	0.174	0.589
	Smoking**	0.291	0.335	-0.357	0.231	0.000	1.000	-0.095	0.758	-0.024	0.937	-0.145	0.636
	Brinkman Index*	-0.102	0.741	-0.112	0.715	0.519	0.069	-0.319	0.288	-0.284	0.347	-0.495	0.085
Maxillary	Age*	0.059	0.872	0.377	0.282	-0.138	0.703	0.482	0.158	0.618	0.057	0.302	0.396
	Gender**,¶												
	Duration*	0.374	0.409	-0.432	0.333	0.605	0.150	-0.816	0.025	0.031	0.948	0.335	0.462
	Smoking**	-0.206	0.658	0.206	0.658	-0.764	0.046	0.471	0.286	-0.471	0.286	0.258	0.576
	Brinkman Index*	-0.651	0.113	0.701	0.079	-0.739	0.058	0.658	0.108	0.473	0.284	-0.303	0.508
Nasal cavity	Age*	0.112	0.759	0.313	0.378	-0.177	0.625	0.291	0.415	0.417	0.230	0.601	0.066
	Gender**	0.268	0.455	-0.621	0.056	0.000	1.000	0.108	0.767	0.481	0.159	-0.048	0.895
	Duration*	-0.189	0.685	0.029	0.951	-0.153	0.743	-0.005	0.991	0.053	0.910	0.255	0.582
	Smoking**	-0.083	0.860	0.163	0.728	0.249	0.591	-0.400	0.374	0.171	0.714	-0.548	0.203
	Brinkman Index*	-0.266	0.565	0.399	0.376	0.415	0.354	-0.376	0.405	-0.016	0.973	-0.515	0.237

Analyzed by Pearson's Correlation Test. **Analyzed by Spearman's Correlation Rho Efficient. ¶In maxillary sinus samples, all of the chosen samples were taken from the male subjects and statistical analysis could not be performed for the gender.

Table V. Correlations between age, gender, polyp duration, smoking and Brinkman Index; and MMP9 and TIMP-1 positivity index levels in the inflammatory cells of the ethmoid sinus, maxillary sinus and nasal cavity.

	MMP9 Positivity Index						TIMP1 positivity index					
	Granulocyte-MMP9-PI		Monocyte-MMP9-PI		Mast cell-MMP9-PI		Granulocyte-TIMP1-PI		Monocyte-TIMP1-PI		Mast cell-TIMP1-PI	
	z	p	z	p	z	p	z	p	z	p	z	p
Ethmoid												
Age*	0.480	0.060	0.331	0.210	0.519	0.040	0.171	0.527	0.130	0.632	0.336	0.204
Gender**	0.153	0.572	-0.216	0.421	0.132	0.625	0.255	0.341	-0.655	0.006	-0.347	0.189
Duration*	-0.398	0.200	-0.021	0.949	-0.133	0.680	-0.334	0.288	0.071	0.826	-0.258	0.419
Smoking **	0.030	0.921	-0.543	0.055	-0.411	0.163	0.184	0.546	-0.426	0.147	0.232	0.445
Brinkman Index*	0.255	0.400	-0.153	0.617	-0.036	0.908	-0.160	0.603	0.084	0.785	0.640*	0.019
Maxillary												
Age*	0.143	0.694	-0.051	0.896	-0.243	0.500	0.235	0.513	0.150	0.678	0.217	0.548
Gender**,¶												
Duration*	0.291	0.527	0.717	0.109	0.304	0.508	0.459	0.300	0.459	0.300	0.296	0.519
Smoking **	-0.354	0.437	-1.000		-0.540	0.211	-0.471	0.286	-0.471	0.286	-0.645	0.117
Brinkman Index*	-0.312	0.496	-0.557	0.251	-0.582	0.171	-0.496	0.257	-0.496	0.257	0.051	0.914
Nasal cavity												
Age*	0.225	0.533	-0.654	0.040	-0.197	0.586	-0.257	0.473	-0.644	0.061	0.269	0.483
Gender**	-0.398	0.254	-0.375	0.286	-0.327	0.356	0.167	0.645	-0.189	0.626	-0.116	0.767
Duration*	-0.255	0.582	-0.656	0.109	-0.569	0.183	-0.374	0.408	-0.165	0.724	0.280	0.543
Smoking **	0.548	0.203	0.300	0.513	0.400	0.374	-0.258	0.576	0.091	0.846	-0.342	0.453
Brinkman Index*	0.515	0.237	0.483	0.273	0.376	0.405	-0.243	0.600	0.269	0.560	-0.224	0.630

*Analyzed by Pearson's Correlation Test. **Analyzed by Spearman's Correlation Rho Efficient. ¶ In maxillary sinus samples, all of the chosen samples were taken from the male subjects and statistical analysis could not be performed for the gender.

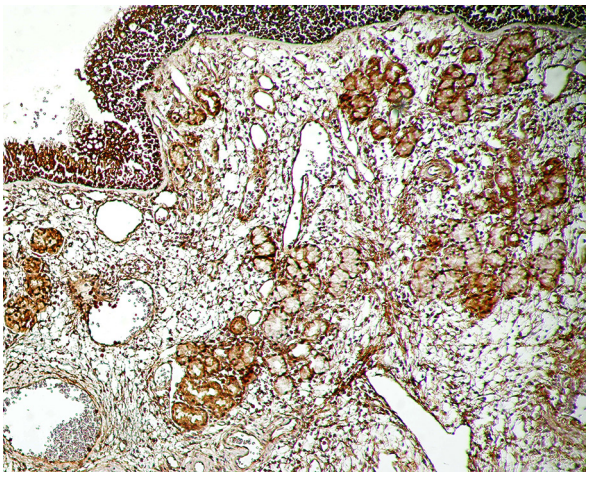

Figure 5. Immunohistochemical staining for MMP2 in polyp sample displaying expression in granulocytes around the blood vessels. Stroma is diffusely edematous. There is intense, diffuse and scattered mononuclear cell infiltration; but in these cells MMP2 positivity cannot be observed (×100).

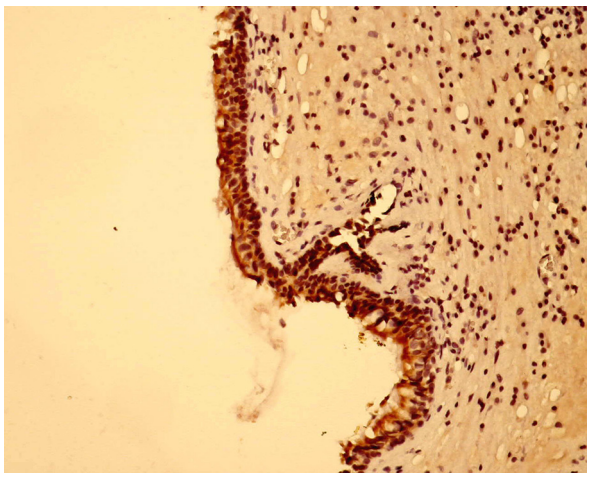

Figure 7. Immunohistochemical staining for TIMP-1 in polyp sample displaying expression in mast cell cytoplasm. In the subepithelial layer, predominantly mononuclear cell infiltration is observed. However, in the mononuclear cells and granulocytes, TIMP-1 expression and staining are absent or minimally positive (×200).

mast cell-PI (MMP2, MMP9) are higher than the rate of TIMP-1-PI of granulocytes and mast cells. The rate of mast cell-TIMP-1-PI was approximately half of the rate of the mast cell-PI (MMP2, MMP9) and was approximately 1/5-1/6 of the granulocyte-PI (MMP2, MMP9). TIMP-1 is the inhibitory enzyme of MMPs. Finally, with higher rate of MMP than the rate of TIMP-1 (MMP/TIMP-1 value increased), the MMPs cannot be inhibited; and net MMP value increases in the cells and tissues. In the present study, especially excessive MMP2 and MMP9, compared to TIMP-1, were primarily detected in the granulocytes, followed by mast cells.

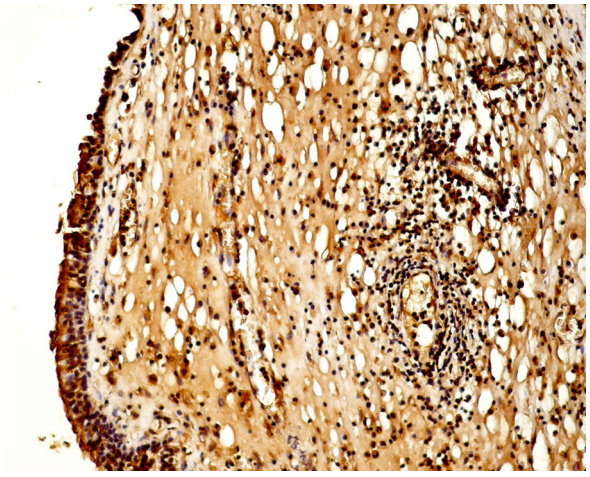

Figure 6. Immunohistochemical staining for MMP9 in polyp sample displaying cytoplasmic MMP9 positivity in granulocytes and mast cells. Both cell groups are dispersed in the subepithelial region and also, more intensely located around the blood vessels of the deep layers of the lamina propria. Intense mononuclear cell infiltration is observed around the blood vessels, whereas in mononuclear cells, MMP9 positivity is observed less. Subepithelial edema is also seen (×200).

The MMP2 and 9 are able to degrade most ECM proteins by their catalytic domain[21-27]. In addition, they can process a large number of non-ECM proteins, such as growth factors, cytokines, chemokines, cell receptors, serine proteinase inhibitors[28]. In our study, we may conclude that with the help of matrix metalloproteinases in granulocyte and mast cells, destruction of the ECM occurs, which might lead to other inflammatory cells to pass through.

Various cell types can store pro- and active MMP2 and MMP9, as well as their activators, in intracellular small exocytic vesicles. These vesicles may be actively propelled along microtubules towards the plasma membrane by the motor protein kinesin. Shedding of such vesicles may be a way of achieving rapid, directional proteolysis during cell migration, invasion or during 3D morphological organization in the process of angiogenesis[5]. This effect may play a role in polyp pathogenesis by inducing angiogenesis; therefore, new inflammatory cells migrate into this region initiating polypoid degeneration process.

Components of ECM are laminin, proteoglycan, fibrillary collagens, elastin and hiyaluronan[29].

Granulocytes contain granules and non-inhibited MMP2 and MMP9; and by the help of these enzymes, ECM is disintegrated. In ECM, spaces or tunnels are created, and thus, a channel is opened for inflammatory cells to migrate and to move in.

Mast cells play a role in IgE dependant hypersensitivity. In the pathogenesis of nasal polyps, they can work in IgE-mediated reactions. Mast cells also have granules in their cytoplasms. Enzymes in these granules and their MMP2 and MMP9 enzymes also work for ECM disintegration and polyp development. Our results were similar to the results of the study by Takasaka et al[9]. They reported that nasal polyps commonly contained many inflammatory cells such as neutrophils (PMN), eosinophils, plasma cells, mast cells, lymphocytes and macrophages. They also suggested that mast cell degranulation plays an important role in the formation of nasal polyps.

Mast cells and basophils contribute to induction and/or maintenance of eosinophilic inflammation by a variety of mechanisms, including IgE-dependent and IgE-independent processes; and they contribute to inflammation both directly through the release of inflammatory mediators, cytokines and growth factors and indirectly through the activation of structural cells; and have particularly important position in the pathogenesis and progression of NP[30]. Degranulated mast cells occurred much more in the deep stroma than in the part near the surface of the polyp; and the pedicle of the nasal polyp had more mast cells and more degranulated ones than did the body and apex. Thus, the pedicle is probably more important in the etiology of polyp formation in nasal mucosa than the body and apex from the viewpoint of the activity of histamine from degranulated mast cells on the blood vessels and nasal glands.

In the present study, monocytes (lymphocytes and plasma cells) are inflammatory cells that occurred more than granulocytes and mast cells in polyp tissue (Figure 1). These cells do not have phagocytosis feature; and their MMP2 and MMP9 contents are very low (Figures 2 and 3). They do not have a role in polyp pathogenesis by the MMPs. These cells increased at the presence of antigenic stimuli, and they migrated into the medium. Plasma cells produced antigen-specific antibodies. By the disintegration of ECM with granulocytes and mast cells, a channel is produced and opened for monocytes to move into the ECM easily. These cells may play a role in polyp pathogenesis through this mechanism.

Nasal secretion of NP patients contained degenerative epithelial cells and neutrophils, activated lymphocytes, monocytes and eosinophils in contrast to normal individuals. Local hyperactivation of T- and B-lymphocytes as well as neutrophils may contribute much into nasal polyp formation[31]. Kong et al[32] reported that the number of eosinophils, CD68 positive cells and monocytes were significantly higher in nasal polyp tissues than in the normal controls. They suggested that infiltration of inflammatory effector cells such as eosinophils and chronic inflammatory changes of the nasal mucosa might play a role in nasal polyps sprouting.

In our study, in the nasal polyp groups, in the older patients, mast cell-MMP9-PI increased in the ethmoid sinus and monocyte-MMP9-PI decreased in the nasal cavity. In the ethmoid sinus, monocyte-TIMP1-PI decreased in males and increased in females. With longer polyp duration, granulocyte-MMP2-PI increased in the ethmoid sinus and decreased in the maxillary sinus. This might have caused less edematous and more fibrotic polyps in the maxillary sinus with prolonged polyp duration.

Increased nuclear gelatinolytic activity, co-localized with MMP-2, has been found[33] in pulmonary endothelial cells undergoing apoptosis. MMP-2 activation in these cells was suggested[33] to be induced by reactive oxygen and nitrogen species produced by cigarette smoke.

In the nasal polyp pathogenesis, MMP increases, and related tissue remodeling may play a role in this. Periostin stimulates gene expression of fibroblasts' MMP-3 and MMP-7-9 in chronic rhinosinusitis. In nasal polyp remodeling, to regulate MMP expression in the epithelial cells and fibroblasts, tenascin C and periostin may be included in the procedure[34].

In our study, in the smoking patients, the mast cell count decreased in the maxillary sinus. The difference of our study from that study may be due to the obstruction of the maxillary sinus ostium by polypoid tissues. Because of the obstruction in the ostium cigarette fume may not have reached the maxillary sinus, and thus, the mast cell counts as well as the mast cell-related MMP2-PI decreased.

Conclusions

We investigated the role of inflammatory cells in nasal polyp pathogenesis. Mast cells and granulocytes have granules, containing matrix metalloproteinases. With the help of

matrix metalloproteinases in granulocyte and mast cells, ECM is destroyed, which might have led to other inflammatory cells to pass through, causing the polypoid degeneration process. Future studies are warranted to decrease MMP/TIMP1 percentages. These investigations may help to decrease MMP values in the tissues, which will help prevent nasal polyp development at various degrees.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

Kirikkale University Faculty of Medicine Local Ethics Committee provided ethics approval (Date: 23.03.2009, No.: 2009/028).

Informed Consent

Retrospective data (data and previously prepared slides in other studies of the authors)[11-15] were used in this study, therefore the informed consent of patients was waived.

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Authors' Contribution

Nuray Bayar Muluk: Planning, designing, literature survey, statistical analysis, interpretation of the results, writing, submission. Osman Kursat Arikan: Planning, designing, data collection, literature survey. Pinar Atasoy: Planning, designing, data collection, literature survey. Rahmi Kilic: Planning, designing, data collection, literature survey. Eda Tuna Yalcinozan: Planning, designing, data collection, literature survey.

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