Evaluation of MMP-9 and TIMP-1 Levels of the Patients with Nasal Polyposis After Corticosteroid Therapies

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Abstract The aim of this study is to evaluate the effects of intrapolyp, systemic and local corticosteroid treatment modalities on MMP-9 and TIMP-1 level in polyp tissues. This study included 71 patients and five groups. Group A treated with oral methylprednisolone, Group B, treated with topical steroid spray, Group C treated with intralesional triamcinolone acetonide, Group D patients with nasal polyposis who has not given any medication, Group E had surgery for concha bullosa without nasal polyp disease. Samples from group A were collected endoscopically 1 day after treatment was stopped. Group B samples were collected at the end of the first month. Video guided sample collection from Group C was also performed on the 7th day after treatment. MMP-9 and TIMP-1 levels were measured by Enzyme-linked immunosorbent assay method. When we compared the MMP-9 levels of all groups, there were no significant differences. There was significant difference of TIMP-1 level between Groups C and E ($p_{C-E} = 0.0019$), however there was no difference among the level of the other groups. When MMP-9/TIMP1 rates of all groups were compared, there were significant difference between Group A and D ($p_{A-D} = 0.005$) and between Group A and E, also between Group C and E. Our

study is the first study to evaluate the effects of different corticosteroid treatment modalities on MMP-9 and TIMP-1 in nasal polyps and concluded that corticosteroid did not do a significant impact on this pathway.

Keywords Nasal polyposis · Matrix metalloproteinase inhibitor · Matrix metalloproteinase-9 · Corticosteroid · Therapy

Introduction

Nasal polyp pathogenesis still remains unclear, but nasal polyposis is considered as an inflammatory pathology in nasal and paranasal sinus cavities [1]. Excessive production and accumulation of the extracellular matrix (ECM) has been reported playing a role in NP formation [2].

Extracellular matrix consists of basement membrane and interstitial matrix, it surrounds and supports the mucosal cells. Balance between synthesis and the degradation of the ECM is very important for homeostasis of cells [3]. The major proteolytic enzyme system responsible for ECM remodeling is comprised of the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). Excessive reduction of ECM occurs with imbalance between MMP and TIMP [4]. MMP-9 (a 92-kDa MMP) degrades almost all the properties of components of the ECM. High rate expression of MMP-9 has been found in chronic rhinosinusitis and NP [4–7]. TIMP-1 is natural inhibitor of MMP-7 and MMP-9 and soluble in water which is located in many tissues, including the nasal mucosa [8, 9]. Especially MMP-2 and MMP-9 are in the main structure of the basal membrane. Because of this feature, the relationship between inflammatory diseases has been examined in further investigations [2, 4, 7, 10].

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Corticosteroids are the most effective treatment modality which are used as topical and systemic treatment of nasal polyps and do significant reduction in symptoms and signs [11]. The effects of systemic or local corticosteroids on the connective tissue are still not fully understood in inflammatory process of nasal polyposis. In several studies, corticosteroids has been shown to inhibit the interstitial collagen synthesis as well as inhibit the production of matrix-degranulating proteinases in many other parts of the body's [12, 13].

This is the first study that has evaluated the effects of intrapolyp, systemic and local corticosteroid treatment modalities on MMP-9 and TIMP-1 levels in polyp tissues.

Subjects and Methods

The study protocol was approved by GATA Medical Faculty (Ankara-Turkey) Clinical Research local ethics committee and informed consent was obtained from each patient. This study included 71 patients with nasal polyposis or concha bullosa. Exclusion criteria were: past surgeries for NP, any glucocorticoid usage for any reason within 1 month, nasal polyp forms other than eosinophilic NP according to pathology study, age less than 15 years, Churg–Strauss syndrome, immunodeficiency, Kartagener's syndrome and Young syndrome, cystic fibrosis, antrochoanal polyp and unilateral nasal polyp. All patients were evaluated for steroid treatment contraindication.

Study Design

The patients were divided into five groups.

Group A ($n = 14$ (19.72 %)), treated with oral methylprednisolone (Prednol® 16 mg tablet, Mustafa Nevzat Pharmaceutical, Istanbul-Turkey) which was applied for 21 days and tapered gradually, with a reduction rate of 16 mg/3 days.

Group B ($n = 13$ (18.31 %)), treated with two puffs for each nostril once daily of topical steroid spray (55 microgram triamcinolone acetonid (Nasacort AQ Spray®, Sanofi Aventis Pharmaceutical, Turkey) for 1 month.

Group C ($n = 13$ (18.31 %)), treated with 0.3 cc 40 mg/ml intralesional triamcinolone acetone (Kenacord-A® 40 mg/ml ampoule, Bristol-Myers Squibb Pharmaceutical, Turkey) which was injected in nasal polyp tissue in both nasal cavities using endoscope guidance. Injections were placed with a 27G, 50 mm dental needle in the central area of well visualized polyps, and this procedure was recorded in order to take samples from the same polyps after treatment.

Group D ($n = 17$ (23.94 %)), positive control group, patients with nasal polyposis who has not given any medication.

Group E ($n = 14$ (19.72 %)), negative control group, had surgery for concha bullosa without nasal polyposis disease.

One patient from Group B and C did not come to clinical examination on time and discarded from the study. In Group D, patients with polyp did not get any medication. Group D gave us valuable information about MMP-9 and TIMP-1 because polyp was not affected from the medication, so we made the objects of this group bigger than those of others.

Tissue Samples

We used three different medication modalities of glucocorticoid (oral medication, topical medication and intralesional injection) and samples were obtained variable time, because their treatment duration was planned particularly depend on their different tissue penetration.

Samples were obtained from the first seen polyp in nasal cavity. In Group A, samples were collected endoscopically 1 day after treatment was stopped so possible complete disappearance of the polyps did not happened. Group B samples were collected at the end of the first month from polyps that were located anteriorly in the nasal cavity. Video-guided sample collection from Group C was also performed on day 7. Group D samples were taken at each patient's first visit to the clinic. Group E samples were taken from medial part of medial turbinate during concha bullosa surgery at operation room endoscopically.

All tissue samples were put into dry tubes and stored in the -80°C freezer until the biochemical assessment.

Biochemical Analysis

After collecting all tissues, they were homogenized by 70 mL tissue in 1 ml 0.9 % NaCl solution. Tissue samples were homogenized with tissue homogenizator. MMP-9 and TIMP-1 levels were measured via ELISA (enzyme-linked immunosorbent assay) method with the human MMP-9 and TIMP-1 Platinum ELISA (eBioscience, Bender MedSystems, Vienna, Austria) Test Kits by using Kayton RT 2100 C microplate reader (Kayton Electronics, China). At the same materials, tissue protein levels were measured by Lowry method. MMP-9 and TIMP-1 concentrations of tissues were calculated as per protein levels of nasal polyp tissues.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 15.0; SPSS Inc., Chicago, IL, USA). Means, modes, medians, standard deviations (SD), proportions and percentages were determined as applicable. The means and SDs were calculated

for continuous variables while ratios and proportions were calculated for categorical variables. Compliance with the normal distribution of the datas was evaluated with Kolmogorov–Smirnov test and graphical representations. Because the datas did not show compliance with the normal distribution, proportions and ratios were compared using Kruskal–Wallis analysis of variance, Mann–Whitney U test, Bonferroni correction ve Chi square test. Within the 95 % confidence interval, $p \leq 0.05$ was considered statistically significant.

Results

This study was completed with 57 patients (49 male, 8 female) with nasal polyposis and 14 patients (13 male, 1 female) without nasal polyposis. Group A included 14, Group B; 13, Group C; 13, Group D; 17 and Group E; 14 patients. No statistically significant difference was found between the groups regarding gender ($p = 0.38$) and age difference ($p = 0.067$). The average age was 36.65. No severe side effects were reported in all groups.

When we compared the MMP-9 levels of all groups, there were no significant differences. And also there was no significant difference among three of each steroid treatment groups (A, B, C) ($p = 0.23$). The distributions of MMP-9 levels within each group are presented in Table 1. There were no significant difference between all treatment groups and control groups ($p_{B-D} = 0.99$, $p_{A-D} = 0.53$, $p_{C-D} = 0.99$, $p_{B-E} = 0.99$, $p_{A-E} = 0.53$, $p_{C-E} = 0.99$).

When we compared the TIMP-1 levels of study groups, there were no significant difference among them (A, B, C) ($p = 0.53$). When we compared between study and control groups, there were no significant difference among them ($p_{B-D} = 0.99$, $p_{A-D} = 0.99$, $p_{C-D} = 0.99$, $p_{A-E} = 0.73$, $p_{B-E} = 0.54$) except between Groups C and E ($p_{C-E} = 0.0019$). The distributions of TIMP-1 levels within each group are presented in Table 2.

When we compared the MMP9/TIMP-1 levels of study groups, there were no significant difference among them (A, B, C) ($p = 0.55$). When three study groups compared

Table 1 The distributions of MMP-9 levels ($\mu\text{g/g}$ protein)

	Mean	SD	Confidence intervals		Min.	Max.
			Lower level	Higher level		
A	57.29	56.71	23.02	91.56	2.43	168.58
B	89.99	69.15	50.07	129.92	15.61	228.99
C	49.31	35.64	27.77	70.85	3.56	140.26
D	63.91	69.57	28.14	99.68	13.85	304.23
E	70.00	40.16	46.81	93.19	6.53	151.19

SD standard deviation, *Min* minimum, *Max* maximum

Table 2 The distributions of TIMP-1 levels ($\mu\text{g/g}$ protein)

	Mean	SD	Confidence intervals		Min.	Max.
			Lower level	Higher level		
A	52.21	55.03	18.96	85.46	5.10	161.94
B	57.52	52.51	27.20	87.84	9.82	194.68
C	32.32	20.88	19.70	44.94	7.81	90.07
D	48.28	50.09	22.52	74.03	9.66	192.58
E	84.94	85.16	35.77	134.11	4.34	357.86

SD standard deviation, *Min* minimum, *Max* maximum

Table 3 The distributions of MMP-9/TIMP-1 levels ($\mu\text{g/g}$ protein)

	Mean	SD	Confidence intervals		Min.	Max.
			Lower level	Higher level		
A	1.38	0.75	0.93	1.83	0.08	2.16
B	1.65	0.19	1.53	1.76	1.18	1.97
C	1.62	0.63	1.25	2.00	0.08	3.08
D	1.42	0.33	1.26	1.59	0.41	1.60
E	1.14	0.54	0.83	1.45	0.22	1.74

SD standard deviation, *Min* minimum, *Max* maximum

with two control groups, there were significant difference between Group A and D ($p_{A-D} = 0.005$) and also between Group A and E, and also between Group C and E ($p_{A-E} = 0.002$; $p_{C-E} = 0.022$). The distributions of MMP-9/TIMP-1 levels were shown in Table 3.

Discussion

Nasal polyp is benign degeneration of nasal and paranasal sinus mucosa with a 4 % of prevalence at adult population [1]. Etiopathogenesis of NP is still unclear. Histologically, the presence of large amounts of extracellular fluid, mast cell degranulation and eosinophil has been shown [14, 15]. In some studies, the excessive accumulation of ECM has been suggested as a role of nasal polyposis [2]. MMP and TIMP can be found in the extracellular tissue, and are inflammatory mediators. These endopeptidases are the most important proteolytic system that creates the renewal of the ESM [14, 16].

Pawankar et al. [17] has found high levels of MMP-9, but low levels of MMP-2 and MMP-13 in NP tissue. Other studies have also shown increased levels of MMP-9 in nasal polyps [2, 4, 5, 7, 18]. Unlike other studies, we could not find statistically significant differences between MMP-9 levels of nasal polyp tissue and healthy nasal tissue.

The most important inhibitor of MMP-9 activity is TIMP-1 [19, 20]. Different results have been reported in nasal polyps for TIMP-1 levels. Kahveci et al. [7] and

Watelet [4] reported of high levels of TIMP-1 but Can et al. [2] found low levels in comparative studies between TIMP-1 and the control groups in patients with NP. However, Chen et al. [18] found no difference in a comparative study. Kostamoa et al. [6] did not find a correlation between TIMP-1 and MMP-7, MMP-8 and MMP-9 in patients with NP. In our study compatible with Chen et al. [18], there were no statistically significant differences between TIMP-1 level of NP and healthy nasal tissue.

Lee et al. [19] also claimed that ratio of MMP-9/TIMP-1 imbalance has a role of imbalances in inflammatory event at nasal polyps. Kahveci et al. [7] reported that MMP/TIMP imbalance may cause a rupture of the epithelia so ECM proteins has a key task for the collapse of the epithelial ruptures.

Toi et al. [20] reported that MMP-9 and TIMP-1 plays a role in angiogenesis and in addition, another study stated that the imbalance between MMP and TIMP increases the vascularisation of nasal polyp [7]. Nevertheless in our study, we evaluated the MMP-9/TIMP-1 values of the groups, and we found statistically significant difference between oral steroid treated group (Group A) and positive control groups (Group D), and between oral steroid treated group (Group A) and negative control group (Group E), and also there were statistically significant difference between intralesional steroid treated group (Group C) and negative control group (Group E).

For many years, corticosteroids have been the main treatment modality for NP. The mechanism contains activating intracellular glucocorticoid receptors and downregulation of the inflammatory protein-coding genes [11]. Reducing the volume of polyps and improving the nasal symptoms have been shown as the effects of corticosteroids [11].

In our study, the differences of oral, intralesion and topical intranasal steroid therapy were not significant statistically for MMP-9 and TIMP-1 levels. However when TIMP 1 levels were evaluated, there was only statistically significant difference between the intralesional and negative control groups. Yigit et al. [10] reported that the use of systemic corticosteroids significantly reduced the level of MMP-2 but did not effect on MMP-9 and TIMP-1. In our study, we evaluate MMP-9 level in steroid treated groups and untreated groups and also healthy nasal mucosa, as a result we could not find any difference among them regarding MMP-9 levels unlikely many other studies [2, 4, 5, 7, 17, 18]. We suggested that MMP-9 is not significant for nasal polyps and we could not find exact role of corticosteroid treatment modalities on this part of ECM remodeling process.

Yuan et al. [21] reported that dexamethasone prevents airway remodeling by downregulating MMP-2 and TIMP-1 and provides the balance between them. Tanaka et al. [13] reported that methylprednisolone reduced the rate of

MMP-9/TIMP-1 in patients with asthma. In addition, Hoshino et al. [22] reported that by reducing MMP-9 and increasing TIMP-1, corticosteroid treatment decreased the accumulation of subepithelial collagen in asthmatics. In another study [23] showed that inhaled budesonide normalized MMP-8/TIMP-1 ratio by reducing MMP-8 and increasing TIMP-1 in asthmatic children. In contrast to all above studies, all steroid treatment modalities had no effect on the levels of MMP-9 and TIMP-1 in our study. However we found some difference among the groups regarding MMP-9/TIMP-1 ratio.

Kylmaniemi et al. [12] reported that corticosteroid decreases MMP-2 but TIMP-1 and MMP-9 have not been affected in gingival fibroblasts. In our study, compatible with Yigit et al. [10] study, TIMP-1 was not statistically different in all treatment groups compared with the positive control group. As a result we thought that a role of MMP-9 in NP pathogenesis have been losing supporter, TIMP-1 still under investigation because of relationship with other MMPs, however MMP-2 and 8 have been gaining supporter for a role of NP pathogenesis.

Our study is the first study to evaluate the effects of different corticosteroid treatment modalities on MMP-9 and TIMP-1 in nasal polyps and concluded that corticosteroid did not do a significant impact on this pathway. New studies are needed to investigate the effects of different treatment modalities on other proteins in the ECM.

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Conflict of interest No conflict of interest.

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