

Matrix metalloproteinases and their impact on sinusal extension in chronic rhinosinusitis with nasal polyps

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Abstract MMPs (metalloproteinases) and their inhibitor TIMP (tissue inhibitor of metalloproteinases) are important in tissue remodeling and have been implicated in nasal polyp pathophysiology. The objective of the present study was to evaluate gene expression of MMP-1, MMP-2, MMP-9 and TIMP-1 gene expression in nasal polyps and compare them with normal nasal mucosa. Since MMPs could induce a more prominent tissue edema, we also assessed if these gene expressions could be related to a more extensive disease. For the experimental study, 30 nasal polyp samples from patients with CRSwNP (Chronic Rhinosinusitis with Nasal Polyposis) and 19 middle turbinates (MT) from controls were obtained. Gene expression of MMP-1, MMP-2, MMP-9 and TIMP-1 was assessed by qRT-PCR. The expressions of these genes were compared between nasal polyps and controls and correlated to each individual Lund–Mackay score. MMP-1, MMP-2 and MMP-9 were significantly more expressed in nasal polyps than in controls ($P < 0.005$, $P < 0.0001$ and $P < 0.05$ respectively). No expression of TIMP-1 mRNA was detected. There was no correlation between disease extension evaluated by the Lund–Mackay score and MMPs gene expression. The present results demonstrate that there is a significantly higher mRNA gene expression of MMP-1, MMP-2 and MMP-9 in patients with CRSwNP than

controls. However, no correlation was observed between MMPs gene expression and sinonasal CT scan extension. Level of Evidence: 2c

Keywords Nasal polyps · Metalloproteinases · MMP · TIMP · Sinusal mucosa · Chronic Rhinosinusitis

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) are characterized by prominent edema of the sinonasal mucosa, associated with inflammatory recruitment and local tissue degradation [1]. Some studies have suggested that significant changes in mucosa, such as basement membrane thickening, glandular hyperplasia and edema occur due to an extracellular matrix (ECM) protrusion through an epithelial defect [2]. Degradation of ECM proteins is regulated by matrix-metalloproteinases (MMP), a group of enzymes that collectively degrade most of the ECM components and thus induce persistent tissue trauma [2]. The activity of MMPs is counter balanced by the tissue inhibitor of metalloproteinases (TIMP). MMPs and TIMP are secreted by a wide variety of cells, including epithelial cells, fibroblasts and inflammatory cells such as macrophages and neutrophils in response to a noxious stimulus [1].

Several studies have reported the expression of MMPs and their inhibitors (TIMPs) in chronic inflammatory diseases of the airways and their induction after viral and bacterial infections [3]. In addition, drugs such as corticosteroids and macrolides have been shown to decrease MMP-2 and MMP-9 expression in nasal polyps [4].

Despite different efforts, the real role of MMPs in tissue remodeling of the nasal mucosa and its implication on nasal polyps have not been completely elucidated. Thus,

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the objective of the present study was to assess MMP-1, MMP-2, MMP-9 and TIMP-1 gene expression of in nasal polyps, and to correlate their expression to the extent of sinonasal diseased patients with CRSwNP.

Materials and methods

Thirty patients with CRSwNP who underwent endoscopic sinus surgery between 2003 and 2006 at the Division of Rhinology were enrolled. The nasal polyps samples were compared to control samples, i.e. 19 middle turbinate (MT) obtained from patients without nasal symptoms, during their aesthetic rhinoplasty. The study was approved by the Research Ethics Committee (protocol no. 8897/09) and all patients gave written informed consent prior to their participation. Patients younger than 15 years, with unilateral nasal polyps or with associated diseases, such as cystic fibrosis, inverted papilloma and ciliary dyskinesia were excluded from the study.

Immediately before the surgical procedure, we collected patient data concerning age, gender, smoking, asthma and intolerance to aspirin. In addition, CT scan scores were obtained, according to the Lund–Mackay scale [5]. We used the post-operative CT score to make the correlations with the gene expression.

RNA extraction and cDNA synthesis

During surgery, a biopsy of the polyp or MT was obtained, placed in Tryzol[®] solution and kept at -20°C until mRNA extraction. The mRNA was extracted with Tryzol Reagent[®] (Invitrogen), quantified in each tube with a Thermo Scientific[®] Nanodrop 1000 spectrophotometer, and stored at -70°C .

The cDNAs of each sample were obtained using the Super Script[™] III Reverse Transcriptase kit (Invitrogen, Carlsbad, CA, USA), and the reaction was performed according to the manufacture's instruction. The mixture was heated to 42°C for 1 h. The final solution was diluted into 1:10 and this cDNA solution was stored at -20°C .

Quantitative RT-PCR (RTQ-PCR)

The primers were obtained from the Primers Express program (Applied Biosystems, Foster City, CA, USA), according to the reaction specifications (sizes from 18 to 24 bases, melting point (T_m) from 57 to 59°C and CG proportion from 40 to 55 %). The amplification product sizes varied between 56 and 81 pairs of bases. The following genes were quantified: MMP-1, MMP-2, MMP-9 and TIMP-1, besides the housekeeping β -actin, and they are detailed in Table 1.

The RTQ-PCR reactions were performed in duplicate with Platinum[®] SYBR[®] Green qPCR Super Mix-UDG reagent with ROX (Invitrogen) using the ABI PRISM 7000 Sequence Detection System equipment (Applied Biosystems).

The CT values (cycle threshold) for each reaction were obtained, and the results were analyzed by means of the $2^{-(\Delta\Delta C_t)}$ equation.

Statistical analysis

The GraphPad-Prism 5.0 software was used for statistical analysis. All reactions were analyzed and compared by the unpaired Student's *t* test, with the level of significance set at $P < 0.05$. Spearman correlation was used to observe the relation between gene expression and CT score, with the level of significance set at $P < 0.05$.

Results

Patient age ranged from 16 to 71 years, with a mean of 44.2. Fourteen patients were males and sixteen were females. Preoperative endoscopic staging showed that 15 (50 %) patients were in stage 2, while 9 were in stage 1 and 6 patient were in stage 3. Mean Lund–Mackay [6] CT staging obtained was 18/24.

MMP-1 expression was detected in 28 out of 30 NP samples, ranging from 0.21 to 41.5 (mean 9.79 ± 12.62) and in 18 of the 19 MT samples, ranging from 0.09 to 3.88 (mean 1.38 ± 1.21). MMP-1 was significantly more expressed in nasal polyps when compared to controls ($P < 0.005$) (Fig. 1).

MMP-2 gene expression was detected in 26 NP samples, ranging from 1.01 to 7.81 (mean 3.43 ± 1.86) and in 15 MT samples, ranging from 0.06 to 2.4 (mean 0.83 ± 0.61). MMP-2 was significantly more expressed in nasal polyps when compared to controls ($P < 0.0001$) (Fig. 2).

Table 1 Primers employed in RTQ-PCR

Primer	Sequence 5'–3'	Tam amplicon
β -actin	F: TGACTCAGGATTTAAAACTGGAA (24) R: GCCACATTGTGAACCTTTGGG (20)	81
MMP-1	F: GATTGAAAATTACACGCCAGAT (22) R: CTCAATGGCATGGTTCCA (18)	56
MMP-2	F: ACTGTTGGTGGGAACCTCAGA (20) R: TTGTTGCCAGGAAAGTG (18)	62
MMP-9	F: TCACTTCTCTGGGTAAGGAGTA (22) R: TGTCAAAGTTCGAGGTGGTG (21)	90
TIMP-1	F: AACAGCCTGAGCTTAGCTCA (20) R: CATTCCTCACAGCCAACAGT (20)	65

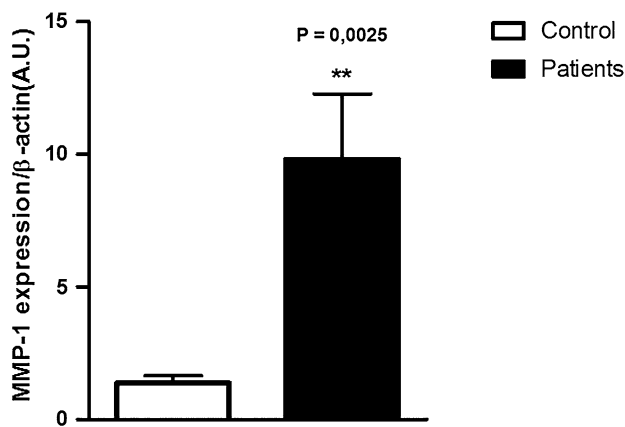


Fig. 1 MMP-1 mRNA expression by RTQ-PCR, using specific primers. Data representing the relative expression between MMP-1 and the β -actin gene (** $P < 0.05$). AU arbitrary units

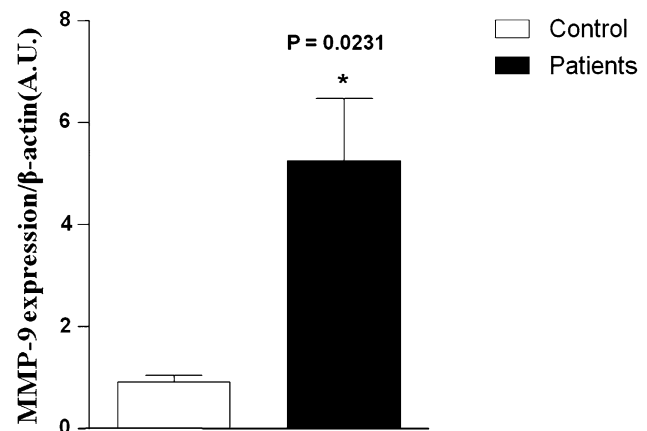


Fig. 3 MMP-9 mRNA expression by RTQ-PCR, using specific primers. Data representing the relative expression between MMP-9 and the β -actin gene (** $P < 0.05$). AU arbitrary units

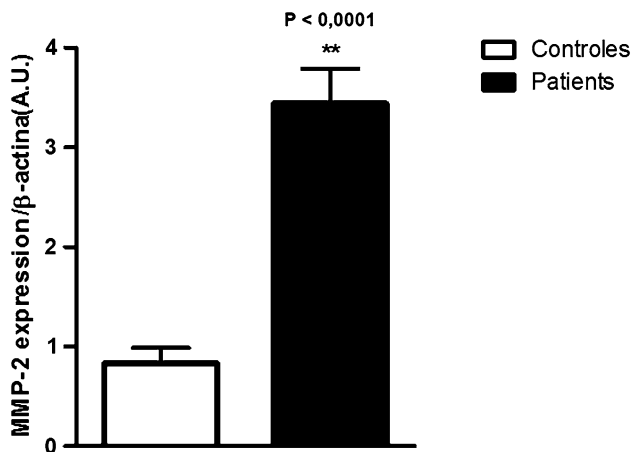


Fig. 2 MMP-2 mRNA expression by RTQ-PCR, using specific primers. Data representing the relative expression between MMP-2 and the β -actin gene (** $P < 0.05$). AU arbitrary units

MMP-9 gene expression was detected in 25 NP samples, ranging from 0.26 to 19.49 (mean 5.25 ± 6.12) and in 16 MT samples, ranging from 0.03 to 1.97 (mean 0.90 ± 0.56). MMP-9 was significantly more expressed in nasal polyps when compared to controls ($P < 0.023$) (Fig. 3).

No expression of TIMP-1 mRNA was detected in patients or controls.

The extensiveness of CRSwNP evaluated by Lund–Mackay CT score did not correlate to MMP-1 ($r = 0.21$, $P = 0.34$), MMP-2 ($r = 0.24$, $P = 0.24$) or MMP-9 ($r = -0.04$, $P = 0.84$).

Discussion

MMPs and TIMPs play an essential role in tissue remodeling. Increased expression of MMPs or reduced TIMP-1

could induce ECM rupture and promote deposit of substances such as water and albumin. This extracellular matrix remodeling could result in edema and local inflammatory process [2], and it would be reasonable to hypothesize that MMPs expression could be related to a more pronounced edema and consequently larger disease extension. Li et al. [6] reported that MMPs and TIMP expressions are different between CRSwNP patients and those with CRS without NP, suggesting that MMPs could be pivotal in worsening wound healing and promoting tissue edema in nasal polyps.

In the present study we compared the gene expression of MMP-1, MMP-2, MMP-9 and TIMP-1 mRNA in CRSwNP patients with controls. We observed that CRSwNP presented higher expression of MMP-1, MMP-2 and MMP-9 than MT controls, but not TIMP-1.

Li et al. [6] reported that MMP-9 would preferentially degrade gelatin, elastin and collagens, and it is likely associated to airway remodeling in CRSwNP. The increase in MMP-9 has already been extensively demonstrated in patients with CRSwNP [6–10].

On the other hand, the role of MMP-2 in CRSwNP continues to be controversial in the literature. Our results demonstrated a higher expression of MMP-2 in nasal polyps when compared to the normal mucosa, in accordance to Bhandari et al. [11]. Instead, other authors have not found an increased expression of MMP-2 in nasal polyps [1, 6, 12]. This disagreement in literature results may be explained by the different techniques employed in each study.

We observed a higher expression of MMP-1 mRNA in patients with nasal polyps when compared to controls, suggesting that MMP-1 (also known as collagenase) could play an important role in CRSwNP. MMP-1 expression had not been previously studied in nasal polyps, although Van

Bruaene et al. [13] observed significant lower concentrations of collagen in nasal polyps when compared to controls and to chronic rhinosinusitis without nasal polyps, and this could be related to the increase of MMP-1 expression.

Watelet et al. [8] were the first to confirm, by means of immunohistochemistry, the presence of TIMP-1 in nasal polyps, although they have observed similar levels between nasal polyps and the nasal mucosa from controls, such as other authors [7, 9]. In contrast, Can et al. [10] observed a significantly higher expression of TIMP-1 in nasal polyps when compared to controls. In the present study, we could not detect TIMP-1 mRNA expression neither in nasal polyps nor in controls.

As metalloproteinases degrade ECM and increase albumin and water deposition in nasal polyps, we have also speculated that MMPs expression could be related to the extent of the sinus disease. Interestingly, Watelet et al. [14] have observed that a higher expression of MMP-9 in nasal polyps was associated with poor post-operative healing. We decided to use the CT scan score, because the endoscopic scoring considers only the nasal extension of nasal polyps and its classification is less sensitive than the former. To the best of our knowledge, no studies had correlated the MMP expression to the extension of sinus disease so far. Our results suggest that the increased expression of MMPs is not correlated with the sinonasal CT scan findings. However, since Lund–MacKay score classifies sinonasal mucosal thickness between 0 and 2, minimal histological remodeling promoted by MMPs and TIMP could not be quantified by a gross CT evaluation.

Both macrolides [15] and glucocorticoids [4] have shown anti-inflammatory properties on nasal mucosa suppressing the activity of both MMP-2 and MMP-9 [15]. Hypothetically, the clinical improvement promoted by these two drugs in CRS could be related to a decrease in MMPs expression.

Further studies involving MMPs and TIMPs in CRSwNP are essential to understand this pathophysiology mechanism in nasal polyps and to make possible newer therapeutic options for these patients.

Conclusions

We may conclude that MMP-1, MMP-2 and MMP-9 expressions, but not that of TIMP-1, were higher in patients

with CRSwNP when compared to controls. MMPs expression was not correlated to disease extension.

Conflict of interest None

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