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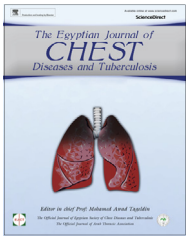

ORIGINAL ARTICLE

Asthma remodeling: The pathogenic role of matrix metalloproteinase-9

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KEYWORDS

Asthma remodeling;
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Abstract *Background:* Asthma is an airway inflammatory disease with functional and structural changes, leading to bronchial hyperresponsiveness and airflow obstruction. Pathological repair of the airways leads to these structural changes referred as airway remodeling. Matrix metalloproteinases (MMPs) are extracellular degrading enzymes that play a critical role in the remodeling process.

Aim of the study: Is to study matrix metalloproteinase-9 in asthmatic patients, detecting its pathogenic role in airway remodeling.

Subjects and methods: Samples of broncho-alveolar lavage (BAL) fluid and bronchoscopic biopsies from 30 asthmatic patients (10 mild, 10 moderate and 10 severe) and 10 healthy volunteers were assessed for the levels of matrix metalloproteinase-9 (MMP-9) total and differential cell count (in BAL fluid), histological airway remodeling changes and immunohistochemical expression of MMP-9 (in mucosal biopsies).

Results: BAL and tissue MMP-9 (going hand in hand with airway remodeling changes) were higher in asthmatic patients and it was significantly increased with increased severity. BAL total cell count is higher in asthmatic patients. BAL eosinophils, neutrophils, lymphocytes as well as MMP-9 positive cell count were higher in asthmatic patients and increased with severity. MMP-9 tissue expression was also strongly inversely correlated with the spirometric parameters in asthmatic patients.

Conclusions: MMP-9 plays a role in airway inflammation and airway remodeling in asthma. MMP-9 is an important player in airway remodeling in bronchial asthma and may be the link between inflammation and remodeling processes.

Introduction

Asthma is a chronic disease of the lower airways characterized by airway inflammation, reversible airflow limitation, and bronchial hyperresponsiveness (BHR) [1].

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Deaths from asthma continue to occur in spite of our increased understanding of the pathophysiology of asthma, the availability of more effective medications for the control of airway inflammation and improved asthma education [2].

Such patients who die from asthma demonstrate thickened airway walls due to an increase in smooth muscle mass, infiltration with inflammatory cells, deposition of connective tissue, vascular changes and mucous gland hyperplasia, a condition that termed airway remodeling [3,4].

Mechanisms controlling the pathogenesis of airway remodeling are poorly understood. One of such mechanisms is the imbalance between extra cellular matrix (ECM) production and collagen degradation [5].

Matrix metalloproteinases (MMPs) are enzymes playing central roles in the turnover of ECM components as well as tissue degradation, repair mechanisms and cell migration. Matrix metalloproteinase-9 (MMP-9) is a 92-kDa metalloproteinase also known as gelatinase B. It belongs to a group of gelatinases that has received special interest because of their ability to degrade elastin and also type IV collagen [6].

Although many intrinsic lung cells can be stimulated to produce MMP-9, inflammatory cells are thought to be the primary source of MMP-9 in disease [7,8].

Aim of the work

The aim of this work was to study matrix metalloproteinase-9 in asthmatic patients, detecting its role in the pathogenesis of airway remodeling.

Subjects and methods

This study was carried out on 30 asthmatic patients and 10 non-smoker healthy volunteers (control group). Subjects of the study were selected from patients who admitted in the Chest Department, Tanta University Hospitals during the period from January 2012 to May 2013.

Subjects were classified into four groups; 10 healthy individuals as control (Group I), 10 patients with mild persistent asthma who had nighttime symptoms not more than twice per month., FEV1 or PEF > 80% predicted (Group II), 10 patients with moderate persistent asthma who had nighttime symptoms more than once per week but not nightly, FEV1 > 60% but <80% (Group III) and 10 patients with severe persistent asthma who had often nightly symptoms, FEV1 < 60% (Group IV) [7].

Neither the normal control subjects nor the patients with chronic persistent asthma had any history of respiratory infection for at least 4 weeks before the study, and none of the participants smoked. This study was performed with the approval of the Ethics Committee of the Faculty of Medicine, Tanta University Hospital, and informed written consent was obtained from all enrolled subjects.

After thorough history taking and complete physical examination, all subjects underwent Chest X-ray (PA and lateral views), ventilatory function tests (including: FVC, FEV1, FEV1/FVC, and PEFR), bronchoalveolar lavage (BAL) that was collected for total and differential inflammatory cell count, and for the estimation of MMP-9 (using a MMP-9 ELISA kit)[8] and finally, a bronchoscopic biopsy that was taken for histopathological examination and estimation of MMP-9 by immunohistochemistry using a Rabbit anti-human polyclonal antibody against MMP-9 (Ab-9), ((catalog # RB-9234-P), Lab Vision; Fremont, USA) with overnight incubation at 4 °C after microwave antigen retrieval in 10 mM sodium citrate buffer (pH 6.0) [9]. Evaluation of the immunohistochemical results was done by Leica image analysis software and the number of MMP-9 +ve cells was standardized to square millimeter of tissue section surface area [10].

Results

The subjects of our study were classified into four groups. The mean values of age were; 25.1 ± 4.5 (Group I), 36.3 ± 6 (Group II), 38.3 ± 6.4 (Group III), and 33.6 ± 4.8 (Group IV) with no significant differences. Spirometry was done for all subjects, measuring FVC, FEV1, FEV1/FVC, and PEFR (Table 1).

Bronchoalveolar lavage was done for all studied subjects to assess the total inflammatory cell count/mm^3, and percentage of eosinophils and neutrophils, and to assess levels of MMP-9 (ng/ml).

The mean values of BAL total cell count were $157.3 \pm 28.01 \times 10^3$, $158.3 \pm 32.3 \times 10^3$, $176.9 \pm 32.8 \times 10^3$ and $200.2 \pm 35.3 \times 10^3$ in groups I, II, III and IV respectively, with a significant increase in group IV compared with groups I, II and III.

The mean values of the BAL eosinophils% were 0.49 ± 0.14, 0.97 ± 0.36, 1.26 ± 0.37 and 2.08 ± 0.53 in groups I, II, III and IV respectively, with a significant increase in groups II, III and IV compared with group I, in groups III and IV compared with group II and in group IV compared with group III.

The mean values of the BAL neutrophils% were 0.68 ± 0.19, 0.70 ± 0.14, 1.46 ± 0.30 and 2.65 ± 0.41 in groups I, II, III and IV respectively, with a significant increase in groups III and IV compared with group I, in groups III and IV compared with group II and in group IV compared with group III.

The mean values of MMP-9 were 23.7 ± 3.77, 57.6 ± 10.12, 65.4 ± 12.81 and 131.2 ± 21.56 ng/ml in groups I, II, III and IV respectively, with a significant increase in groups II, III and IV compared with group I, and in group IV compared with groups II and III (Table 2).

Bronchial biopsy was taken for both histopathological examination and MMP-9 estimation by immunohistochemistry.

Histopathological examination showed airway structural changes in asthmatic patients, ranging from thickened basement membrane with loss of surface epithelium (epithelial shedding) in patients with mild asthma, to respiratory epithelial hyperplasia, marked basement membrane thickening, hypertrophy and hyperplasia of the airway smooth muscle cells, and hyperplasia of the mucus glands, angiogenesis, and increased collagen deposition in the sub epithelial layer in patients with severe asthma (Fig. 1).

MMP-9 immune reactivity in the lung tissues showed absence of MMP-9 positivity in the epithelium with negative submucosa in group I, mild positivity in the epithelium with negative submucosa in group II (MMP-9 +ve cells/mm^2 = 41.8 ± 4.77), moderate positivity in the epithelium and the underlying structures as the submucosa and the

Table 1 Mean (±SD) values and statistical comparison of age and studied spirometric parameters in the four groups.

	G.I	G.II	G.III	G.IV
Age	25.1 ± 4.5	36.3 ± 6	38.3 ± 6.4	33.6 ± 4.8
f. test	0.635			
P	0.558			
FVC	99.5 ± 5.12	93.8 ± 6.84	90.2 ± 5.88	76.1 ± 10.72
f. test	5.36			
P	0.008			
FEV1	99.9 ± 5.54	89.6 ± 4.24	71.1 ± 4.81	52.6 ± 5.08
f. test	17.523			
P	0.001			
FEV1/FVC	101.3 ± 4.76	83.1 ± 7.90	71.4 ± 6.67	68.2 ± 7.91
f. test	46.702			
P	0.001			
PEFR	103.1 ± 5.87	94.1 ± 6.52	71.6 ± 4.19	59.0 ± 5.35
f. test	132.816			
P	0.001			

Table 2 Mean (± SD) values and statistical comparison of BAL total cell count, eosinophils%, and neutrophils% in the four groups.

	G.I		G.II		G.III	G.IV
Total cell count × 10^3	157.3 ± 28.01		158.3 ± 32.3		176.9 ± 32.8	200.2 ± 35.3
f. test	3.225					
P	0.016					
Tukey's test	G.I&II	G.I&III	G.I&IV	G.II&III	G.II&IV	GIII&IV
	0.945	0.142	0.005	0.255	0.006	0.002
Eosinophils%	0.49 ± 0.14		0.97 ± 0.36		1.26 ± 0.37	2.08 ± 0.53
f. test	6.335					
P	0.001					
Tukey's test	G.I&II	G.I&III	G.I&IV	G.II&III	G.II&IV	G.III&IV
	0.001	0.001	0.001	0.001	0.001	0.001
Neutrophils%	0.68 ± 0.19		0.70 ± 0.14		1.46 ± 0.30	2.65 ± 0.41
f. test	9.336					
P	0.001					
Tukey's test	G.I&II	G.I&III	G.I&IV	G.II&III	G.II&IV	G.III&IV
	0.877	0.001	0.001	0.001	0.001	0.001

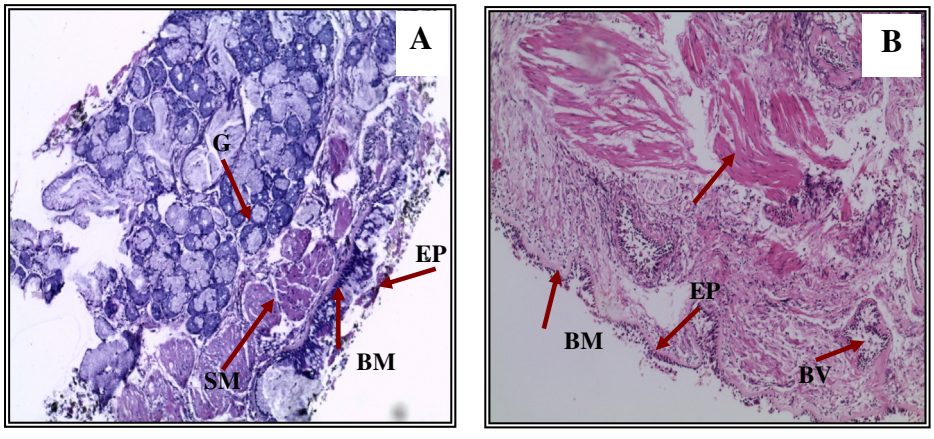

Figure 1 Marked remodeling changes (group IV, severe asthma): [A] respiratory epithelial hyperplasia (EP), thickened basement membrane (BM), hypertrophy of smooth muscle cells (SM), and hyperplasia of the mucus glands (G), [B] other areas showed widening of the submucosa with marked inflammation, angiogenesis (BV), thickening of basement membrane (BM) and partially denuded surface epithelium (EP) (×40; H&E).

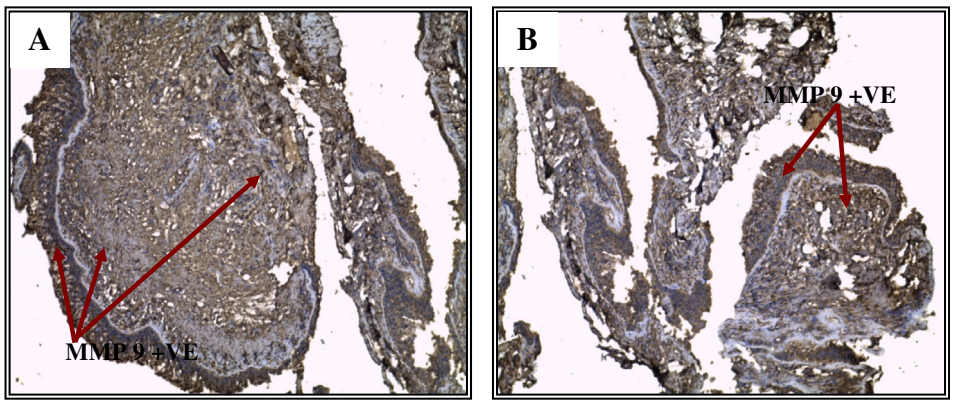

Figure 2 Marked positivity for MMP-9 (Group IV; severe asthma) in the epithelium, submucosa [A and B] and the underlying smooth muscle [A] (×40; immunoperoxidase for MMP-9).

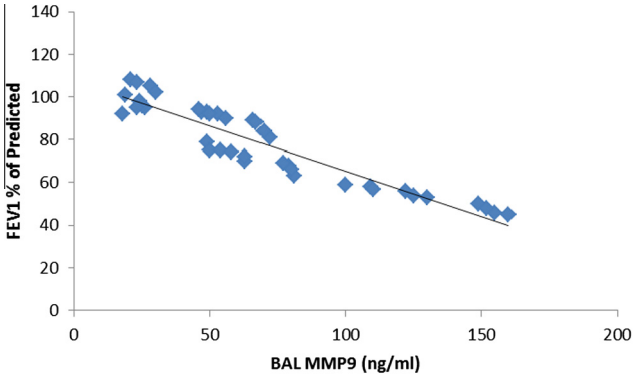

Figure 3 Correlation between FEV1 % predicated and BAL MMP-9 in the studied groups.

underlying muscles, hyperplastic mucous glands and smooth muscles in group III (MMP-9 +ve cells/mm^2 = 57.1 ± 8.74), and marked positivity for MMP-9 in the epithelium, submucosa and the underlying smooth muscle in group IV. (MMP-9 +ve cells/mm^2 = 94.1 ± 11.9) (Fig. 2). MMP-9 +ve cell counts per mm^2 showed a significant difference between groups, being the highest in severe asthma and the lowest in mild asthma ($p < 0.001$). MMP-9 immune reactivity was strongly inversely correlated with spirometric parameters in asthma patient groups ($p < 0.001$).

Correlation of BAL MMP-9 concentration, (ng/ml) and FEV1% predicted in the four studied groups showed a significant negative correlation ($r = 0.764$, $p < 0.05$) (Fig. 3).

Discussion

This work aimed to study matrix metalloproteinases-9 in asthmatic patients, detecting its pathogenic role in airway remodeling.

In this study, we have demonstrated that BAL total cell count is increased in patients with asthma compared with healthy subjects. There was a significant increase in patients with severe asthma compared with mild and moderate asthmatics. Also, in our study there was a significant increase in the BAL eosinophils in patients with asthma compared with healthy subjects. There was a significant increase in patients with severe asthma compared with mild and moderate asthmatics, and in patients with moderate asthma compared with patients with mild asthma. This association between eosinophilia and outcomes of asthma severity has been confirmed and extended in several other studies that have shown that BAL eosinophil numbers are the highest in patients with more severe asthma [11–14].

In this study, there was a significant increase in BAL neutrophils in patients with asthma compared with healthy subjects. Also, there was a significant increase in patients with moderate and severe asthma compared with mild asthmatics. Some studies concluded that increased neutrophil levels have been found in patients with acute or persistent asthma compared with controls, especially in patients with low numbers of eosinophils and poor response to inhaled corticosteroids [15–18].

The present study showed a significant increase in BAL fluid MMP-9 in patients with asthma compared with control subjects and in patients with severe asthma than in patients with mild to moderate asthma. Such increased BAL MMP-9 levels showed a significant positive correlation between MMP-9 concentration and BAL total cell count in patients with asthma, regardless of the degree of severity. Such finding explained that the main cellular sources of MMP-9 are the inflammatory cell infiltrates rather than resident ones.

There was a significant positive correlation between BAL MMP-9 concentration and BAL eosinophils in patients with asthma denoting that asthma is an inflammatory disease mainly eosinophilic in nature.

Also, as a unique finding, there was a significant positive correlation between MMP-9 concentration and neutrophils in BAL only in patients with severe asthma. These data may speculate that neutrophil influx is the main source of increased MMP-9 in patients with severe asthma and deserves further research.

In our study, healthy subjects showed nearly-normal airway structure, but asthmatic patients showed structural airway changes even in patients with mild asthma. This implies that the structural changes of airway remodeling occur at the same time as the onset of airway inflammation. Coinciding with these structural changes, airways showed mild positivity for MMP-9 in the epithelium with negative submucosa in mild asthmatic patients, moderate positivity in the epithelium and the underlying structures as the submucosa and the underlying

muscles, hyperplastic mucous glands and smooth muscles in the moderate asthmatic patients, marked positivity for MMP-9 in the epithelium, submucosa and the underlying smooth muscle in severe asthmatic patients. The MMP-9 immunoreactivity was the highest in the severe asthma group followed by the moderate asthma group and the lowest in the mild asthma group. Moreover MMP-9 tissue immunoreactivity was strongly inversely correlated with the spirometric parameters, a finding that stands with the important role of MMP-9 in airway remodeling. So, targeting MMP-9 in asthma patient may have an important role in delaying or stopping airway remodeling and avoiding its bad consequences on the patient.

Conclusions

Increased inflammatory burden coincides with increased level of proteinases especially MMP-9 in the airways. Increased MMP-9 burden in the airways could result in ECM destruction and contribute to airway remodeling and decline in lung functions in asthmatic patients.

Recommendation

Increased MMP-9 levels in the airways could detect a subset of asthmatic patients with MMP-9 dependent remodeling, allowing early detection and early treatment of these alterations that may be of beneficial value in the prognosis of asthmatic patients.

Conflict of interest

We have no conflict of interest to declare.

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