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ORIGINAL ARTICLE

Asthma remodeling: The pathogenic role of matrix metalloproteinase-9

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KEYWORDS

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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