Egyptian Journal of Bronchology

Vol. 6, No 1, June, 2012

ORIGINAL ARTICLE

IMBALANCE BETWEEN SERUM MATRIX METALLOPROTEINASE-9 AND ITS INHIBITOR IS ASSOCIATED WITH INCREASED AIRWAY WALL THICKNESS IN UNCONTROLLED ASTHMATICS

Aliaë AR Mohamed-Hussein,[1] Samy Abdel Aziz,[2] Arwa Ahmed[3]

[1]Chest, [2]Radiology, [3]Biology Departments, Faculty of Medicine and Science*, Assiut University

Correspondence to: Aliaë AR. Mohamed-Hussein, Email: massah_99@yahoo.com

Abstract: ***The end result of airway inflammation and remodeling is an increase thickness of airway wall, leading to reduction of baseline airway caliber and exaggerated airway narrowing. Current evidences suggest that matrix metalloproteinase- 9 (MMP-9) mediates several important pathways for asthma exacerbation including airflow obstruction, increased vascular permeability and exaggerated airway hyper responsiveness. Moreover the balance between MMP and tissue inhibitor metalloproteinase-1 (MMP-9/TIMP-1 ratio) has been proposed as prognostic indicator in asthma. Objectives: To estimate serum levels of MMP-9, TIMP-1 and MMP-9/TIMP-1 in patients with controlled and uncontrolled asthma then to correlate these serum markers with the parameters of airway wall thickness in HRCT scans of asthmatics. Patients: 52 patients with asthma and 23 healthy controls were recruited in this study. Methods: We measured airway luminal area (Ai) corrected by body surface area (Ai/*** $\sqrt{BSA}$***), airway wall area (WA) corrected by body surface area (WA/*** $\sqrt{BSA}$***), the percentage of wall area (WA %), absolute wall thickness (T)/BSA, and thickness to diameter ratio (TDR) by HRCT. Spirometric tests were also performed. Serum levels of MMP-9, TIMP-1 and their ratio were measured in the serum by ELISA. Results: Serum MMP-9 levels were inversely correlated with WA%, WA/*** $\sqrt{BSA}$***, and TIMP-1 levels were positively correlated with WA/BSA and T/BSA. The MMP-9/TIMP-1 ratio was inversely correlated with WA% and T/BSA and positively correlated with post-bronchodilator values of mid-forced expiratory flow and forced expiratory volume in 1st second. Conclusion: imbalance between MMP-9 and its inhibitor may have a role in airway wall thickening in uncontrolled asthmatic patients and may result is remodeling and decreased reversibility of airflow obstruction.***

Keywords: ***Asthma, remodeling, matrix metalloproteinase-9, tissue inhibitor matrix metalloproteinase-1, pulmonary function test.***

INTRODUCTION

Connective tissue cells produce and secrete an array of macromolecules forming a complex network filling the extracellular space of the sub-mucosa called the extracellular matrix (ECM).(1) The ECM is a dynamic structure, and an equilibrium between synthesis(2) and degradation of ECM components is required for the maintenance of its homeostasis. Although many proteases can cleave ECM molecules, matrix metalloproteinases (MMPs) and their inhibitors are likely to be the normal physiologically relevant mediators of ECM degradation.(3,4) At least three subclasses of MMPs have

been identified: collagenases, gelatinases, and stromelysins. They are mainly synthesized by connective tissue cells, granulocytes, and monocyte macrophages.[5] The major physiologic inhibitors of MMPs are the broad spectrum serum inhibitor a2-macroglobulin and a special class of tissue inhibitors of metalloproteinases (TIMPs).[3] The balance between MMPs and their inhibitors is critical in tissue repair and remodeling, and its homeostasis plays an important role in the breakdown and deposition of ECM in the airways wall.

Asthma is an inflammatory disease of the airways characterized by airways remodeling, due, at least in part, to an excess of ECM deposition in the airway wall,[6,7] which leads to subepithelial collagen deposition.[8] These structural changes of the airways may represent an important cause of airflow obstruction in asthma.[10]

In the present study we evaluated whether in asthma the degree of airflow obstruction and thickness, as assessed by FEV1 values and airway wall thickness parameters in High resolution CT (HRCT), was associated with an imbalance between the serum levels of MMP-9 and its tissue inhibitor, TIMP-1.

PATIENTS AND METHODS

Patients: Fifty- two patients with asthma and 23 healthy control subjects matched for age and sex were selected randomly from the chest department and Out- Patients clinic, Assiut University Hospitals. Asthma was diagnosed and classified according to Global initiative for Asthma (GINA, 2011).[11] Uncontrolled asthma was defined as three or more features of partly controlled asthma present in any week and one exacerbation in any week. Partly controlled patients had daytime symptoms >twice/week, any limitation of activity, nocturnal symptoms/awakening, need for reliever/rescue treatment >twice/week, FEV1 or PEF<80% predicted and one or more exacerbation/year. Controlled asthmatics had none of these criteria and normal lung function. The presence of chronic bronchitis, emphysema, bronchiectasis, and pulmonary tuberculosis were ruled out by history, clinical examination, and chest radiography.

Control subjects were healthy volunteers with no history of asthma and had normal spirometric results, none of them had any record of past or current major disease (heart or lung disease). Pregnancy was an exclusion criterion.

Pulmonary function tests: Spirometric tests were performed with ZAN Spirometer (Messgeraete GmbH, Germany, Model 963) to measure forced vital capacity (FVC), forced expiratory volume in the 1st second (FEV1), FEV1/FVC %. Each measurement was repeated at least three times and the highest acceptable measurement was compared with normal predicted values.

HRCT scanning: The thoracic CT examination was performed with Toshiba X-press scanner. All examinations were performed from the apex to the base of the lungs, with the patient in supine position at end of inspiration (end-inspiratory volume) without use of contrast
medium.

Image evaluation: We assessed the bronchial wall thickness as follows: all visible sections of the bronchi were counted at five levels in both pulmonary fields. All circular (complete circles or at least two thirds of a circle) and longitudinal bronchi except hilar bronchi were included. The five levels were 1 cm above the carina, 1cm below the carina, right pulmonary vein, and 2-3cm below the top of the pulmonary vein and above the right side of the diaphragm. For each patient we computed the total sum of all cross sectional areas of selected bronchi and the airway dimensions were assessed by validated HRCT techniques (Little et al., 2002).[12]

We measured airway wall area (WA) corrected by body surface area (BSA), the percentage of wall area [WA%= { [π $(D/2)^2$ -π $(L/2)^2$]/ $\pi(D/2)^2$ }.100]), absolute wall thickness (T)/$\sqrt{}$BSA, thickness to diameter ratio (TDR) and airway luminal area/BSA .Body surface area was computed using the Dubois formula: BSA(m2)= 0.20247 x height(m) 0.755 x weight (kg) 0.425.

Measurement of MMP-9 and TIMP-1 by ELISA in Serum.

Determinations of the absolute value of MMP-9 and TIMP-1 in serum were performed by enzyme-linked-immunosorbent assay (ELISA) (MMP9-BIOTRAK ELISA and TIMP1- BIOTRAK ELISA; Amersham International plc, Little Chalfont, Buckinghamshire, UK). These assays measure the total amount of each respective protein (MMP-9 and TIMP-1), whether free or complexed to one another or to matrix.

Statistical Analysis: We used SPSS version 11 for windows for statistical analysis of data. The means for groups were compared using the Mann- Whitney's test. Differences between groups and subgroups were assessed by analysis of variance (ANOVA). The Spearman tests were used to determine correlation between data.

Ethical consideration: The Assiut University's Ethics Committee approved the study and all subjects gave written informed consent.

RESULTS

Demographic and HRCT Characteristics of Patients

The sex distribution and mean ages of patients with asthma and of control subjects were similar. FVC, FEV_1 and FEV_1/FVC% values of uncontrolled asthmatic patients were significantly lower than controlled asthmatics and normal controls (Table 1).

The findings of HRCT scan measurements are shown in Table 2. The Ai, Ai/BSA, WA, WA/BSA, WA% and T/BSA, were significantly higher in patients with uncontrolled asthma than controlled asthmatics and healthy controls.

MMP-9 and TIMP-1 Levels in Serum

The concentrations of MMP-9 were significantly greater in serum of uncontrolled asthmatics than in serum of controlled asthmatics and normal control subjects ($p<0.01$, 0.05 Mann-Whitney U test, respectively) (Table 2). TIMP-1 levels were significantly higher in uncontrolled and controlled asthmatics than in control subjects ($p <0.05$, Mann-Whitney U test). The ratio MMP-9/TIMP-1 was significantly lower in uncontrolled asthmatics than in controlled asthmatics and normal control subjects ($p<0.01$, 0.05, and 0.01 respectively, Mann-Whitney U test) (Table 2).

Correlation between MMP-9 and TIMP-1 and its ratio and airway wall thickness parameters

Serum MMP-9 levels were inversely correlated with WA%, WA/$\sqrt{BSA}$, and TIMP-1 levels were positively correlated with WA/BSA and T/BSA. The MMP-9/TIMP-1 ratio was inversely correlated with WA% and T/BSA and positively correlated with post-bronchodilator values of mid-forced expiratory flow and forced expiratory volume in 1st second.

Table 1. Clinical and Functional Characteristics in uncontrolled asthmatics and control group.

Variable	Uncontrolled asthma n= 35	Controlled asthma n=17	Controls n=23	Significance
Gender				
Male, n (%)	19	10	13	NS
Female, n (%)	16	7	10	
Age, years, mean (SD)	46.7 (3.4)	46.7 (19)	44.7(12)	NS
FVC% of predicted, Mean (SD)	46. 3 (7)	95 (3)	101(2)	P1<0.001 P2<0.001
FEV1% of predicted, Mean (SD)	52.7 (3)	94.3 ± 16.6	107.7	P1<0.001 P2<0.00
FEV1/FVC%, Mean (SD)	43.6 (4)	76.9 ± 9.9	81.1 ± 5.3	P1<0.001 P2<0.01

Table 2. Airway wall thickness parameters by HRCT and biochemical parameters in the studied groups.

Variable	Uncontrolled asthma n= 35	Controlled asthma n=17	Controls N=23	Significance
Ai/BSA, mm2/m2, Mean (SD)	13.1 (4.6)	9.2 (4.5)	9.7 (4.1)	P1<0.001 P2=NS P3<0.01
WA/BSA, mm2/m2	22.8 (4.1)	12.4 (3.2)	11.6 (2.0)	P<0.001 P2=NS P3<0.01
TDR, Mean (SD)	0.44 (3.2)	0.34(0.01)	0.32(0.09)	P<0.01 P2=NS P3<0.01
Absolute T/BSA Mm/m2	4.1(2.5)	1.3 (0.4)	1.4(0.3)	P<0.001 P2=NS P3<0.01
MMP-9, ng/ml Mean (SD)	554 (23.1)	460 (21.5)	363.2 (16.5)	P1<0.01 P2<0.05 P3<0.01
TIMP-1 ng/ml Mean (SD)	422 (34.1)	209 (26.9)	105 (24.1)	P1<0.01 P2<0.05 P3<0.05
MMP-9/TIMP-1	1.31 (0.2)	2.20 (1.2)	3.45 (1.5)	P1<0.01 P2<0.05 P3<0.01

Ai = luminal area, BSA= body surface area, WA= wall area, WA%= WA / (WA + luminal area) × 100; T = thickness; TDR= thickness to diameter ratio. Data are expressed in mean (SD). Significance was considered at P-values <0.05. NS: Non-significant. P1=uncontrolled asthma versus controls; P2= controlled asthma versus controls P3= uncontrolled versus controlled asthma.

Table 3. Correlation coefficients (r) between airway wall thickness and clinical and biochemical indices in all asthmatic patients.

Variable	Ai	Ai/BSA	T/BSA	TDR	WA%	WA/BSA
FEV1% of predicted	-0.648**	-0.289*	-0.546**	-0.517**	-0.422**	-0.436**
Ratio	-0.398*	-0.461**	-0.393*	-0.317**	-0.399**	-0.365*
FEF 25-75% of predicted	-0.433**	-0.288*	0.327*	0.218 *	0.258**	-0.544**
Reversibility	-0.769**	-0.381*	-0.409**	-0.582**	- 0. 382*	-0.765**
MMP-9	0.421**	0.650**	0.532**	0.512**	-0.409**	-0.503**
TIMP-1	0.598**	0.498**	0.327*	0.152	0.145	0.322*
MMP9/TIMP	-0.323**	-0.531**	-0.313*	-0.373*	-0.468**	-0.548**

*=P<0.05 **P<0.01

DISCUSSION

This study shows that in serum obtained from uncontrolled asthmatic patients the concentrations of MMP-9 and TIMP-1 are significantly increased as compared with those from control subjects.

Airway wall remodeling in asthma is accompanied by degradation of ECM in addition to synthesis and deposition of new matrix. The results of this study support this concept and demonstrate that MMP-9 is the major metalloproteinase detectable in serum, and that its levels are higher in asthmatic patients than in control subjects.

The findings that MMP-9 activity and TIMP-1expression are increased in the serum of subjects who had uncontrolled asthma compared to healthy subjects and patients with controlled asthma are in accordance with the findings of previous studies.[13-17] Lemjabbar and colleagues[13] found a 10-fold to 160-fold increase in MMP-9 activity in BALF in patients receiving mechanical ventilation for the treatment of acute severe asthma over that of non-asthmatic subjects. Mattos et al.,[14] also demonstrated increased MMP-9 activity in the induced sputum of patients who had severe asthma compared with patients who had mild asthma and healthy subjects, although levels of MMP-9 in BALF were not different. Patients who had nocturnal asthma had a fourfold circadian increase in levels of MMP-9 in BALF compared with healthy subjects and asthmatic patients who did not have nocturnal asthma.[18]

Moreover, asthma is characterized by ECM remodeling caused by increased collagen deposition which can lead to airway wall thickening. Although the mechanisms underlying these changes are not completely known, it is likely that in asthma the remodeling of ECM is characterized by an imbalance between metalloproteases and their inhibitors which determines an excessive collagen deposition in the airways.[19]

The results of the present study support this hypothesis and show that in serum of asthmatic subjects the concentrations of TIMP-1 are significantly increased as compared with those of control subjects. The high levels of TIMP-1 may potentially contribute to the pathogenesis of the increased thickness of the basement membrane in asthma.[8]

In addition, in asthmatic subjects the molar ratio between MMP-9 and TIMP-1 is significantly lower than in control subjects, suggesting the existence of a protease–antiprotease imbalance in these diseases. High concentrations of TIMP-1 can lead to an increased deposition of ECM components, and may increase myofibroblast proliferation acting through its cell growth promoting activity.[20]

Similar to the results of the present study, Matsumoto et al., 2005[21] showed that a reduction in the MMP-9/TIMP ratio was associated with worsening airflow obstruction in asthma and increased airway wall thickness, suggesting that lower MMP-9/TIMP-1 ratios may be associated with

a profibrotic environment with reduced extracellular matrix turnover leading to airway remodeling. In contrast, Lao et al., 1999 in one study has shown that the MMP-9/TIMP-1 ratio and specific MMP-9 activity is increased in asthma.[22]

Furthermore, many authors stated that in asthma the inverse correlation between MMP-9/TIMP-1 molar ratio and airway obstruction as well as the higher TIMP-1 concentrations in obstructed than non-obstructed asthmatic patients, support the concept that MMP-9/TIMP-1 imbalance may have important consequences for airway obstruction, probably because of collagen deposition and the increased airway wall thickness.[17-19]

Finally, the evaluation of matrix metalloproteases and of their inhibitors has also been proposed in other recent studies, suggesting that this approach, which is certainly indirect, may be useful for the measurement of markers of airway inflammation and remodeling.[23-24]

In conclusion, this study shows that asthma is characterized by an imbalance between MMP-9 and TIMP-1 which may play an important role in the pathogenesis of tissue remodeling and of airway obstruction.

REFERENCES

1. Alberts B, D Bray, J Lewis, M Raff, K Roberts, J Watson. Cell adhesion, cell junctions, and the extracellular matrix. In B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, editors. Molecular Biology of the Cell, 2nd ed., Garland Publishing Co., New York. 1989:792–834.

2. Mosher D, J Sottile, C Wu, J McDonald. Assembly of extracellular matrix. Curr. Opin. Cell Biol. 1992;4:810–18.

3. Murphy G, AJ Docherty. The matrix metalloproteinases and their inhibitors. Am. J. Respir. Cell Mol. Biol. 1992;7:120–5.

4. Matrisian LM. The matrix-degrading metalloproteinases. Bioessays. 1992;14:455–63.

5. Campbell EJ, JD Cury, SD Shapiro, GI Goldberg, HG Welgus. Neutral proteinases of human mononuclear phagocytes: cellular differentiation markedly alters cell phenotype of serine proteinases, metalloproteinases, and tissue inhibitor of metalloproteinases. J. Immunol. 1991;146:1286–93.

6. Bousquet J, P Chanez, JY Lacoste, R White, P Vic, P. Godard, and F. B. Michel. Asthma: a disease remodeling the airways. Allergy. 1992 47:3–11.

7. Cosio M, H Ghezzo, J Hogg, R Corbin, M Loveland, J Dossman, P. Macklem. The relations between structural changes in small airways and pulmonary function tests. N. Engl. J. Med. 1977;298:1277–81.

8. Roche WR, R Beasley, JH Williams, ST Holgate. Subepithelial fibrosis in the bronchi of asthmatics. Lancet. 1989;1:520–4.

9. Thurbeck WM. Pathology of chronic airflow obstruction. Chest. 1990;97:6S–10S.

10. Wilson JW, X Li, MC Pain. The lack of distensibility of asthmatic airways. Am. Rev. Respir. Dis. 1993;148:806–9.

11. World Health Organization/National Heart, Lung, and Blood Insitute. Global strategy for asthma management and prevention (GINA). Bethesda, MD: National Institutes of Health, National Heart, Lung and Blood Institute, 2011;Publication No. 02–3659.

12. Little SA, Sproule MW, Cowan MD, et al. High resolution computed tomographic assessment of airway wall thickness in chronic asthma: reproducibility and relationship with lung function and severityThorax. 2002;57:247-53.

13. Lemjabbar H, Gosset P, Lamblin C, et al. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. Am J Respir Crit Care Med. 1999;159:1298–307.

14. Mattos W, Lim S, Russell R, et al. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. Chest. 2002;122:1543– 52.

15. Cundall M, Sun Y, Miranda C, et al. Neutrophil-derived matrix metalloproteinase-9 is increased in severe asthma and poorly inhibited by glucocorticoids. J Allergy Clin Immunol. 2003;112:1064–71.

16. Wenzel SE, Szefler SJ, Leung DY, et al. Bronchoscopic evaluation of severe asthma: persistent inflammation associated with high dose glucocorticoids. Am J Respir Crit Care Med. 1997;156:737–43.

17. Jatakanon A, Uasuf C, Maziak W, et al. Neutrophilic inflammation in severe persistent asthma. Am J Respir Crit Care Med. 1999;160:1532–9.

18. Pham DN, Chu HW, Martin RJ, et al. Increased matrix metalloproteinase-9 with elastolysis in nocturnal asthma. Ann Allergy Asthma Immunol. 2003;90:72–8.

19. Fanny W, Diba C, Rotth M, et al. A comparison of airway and serum matrix metalloproteinase-9 among normal subject, asthmatic patients, and patients asthmatic patients with mucus hypersecretion. Chest. 2005;127:1919-27.

20. Hayakawa, T. Tissue inhibitors of metalloproteinase and their cell growth-promoting activity. Cell Struct. Funct. 1994;19:109–14.

21. Matsumoto H, Niimi A, Takemura M, et al. Relationship of airway wall thickening to an imbalance between matrix metalloproteinase-9 and its inhibitor in asthma. Thorax. 2005;60:277–81.

22. Yao PM, Lemjabbar H, D'Ortho MP, et al. Balance between MMP-9 and TIMP-1 expressed by human bronchial epithelial cells: relevance to asthma. Ann NY Acad Sci. 1999;878:512–4.

23. Bossé M, J Chakir, LP Rouabhia, M Audette, G Laflamme, R Laliberté, G Ross, M Laviolette. Serum MMP-9/TIMP-1 ratio as a potential predictive marker of clinical response to oral steroid therapy in severe asthma (abstract). Am. J. Respir. Crit. Care Med. 1998;157:A503.

24. Cataldo D, R Louise, B Duysinx, JM Lewalle, A Noel, JM Foidart, M Radermecker. Increased levels of gelatinase B (MMP9) in the sputum of COPD and asthmatics (abstract). Am. J .Resp Crit Care Med. 1998;102:239-42.