Journal of Pulmonary & Respiratory Medicine

Hassan, et al., J Pulm Respir Med 2015, 5:3
DOI: 10.4172/2161-105X.1000266

Research Article Open Access

# Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) as Non-Invasive Biomarkers of Remodelling in Asthma

**Neveen Abd El-Moneum Hassan[1], Aliaë Abd-Rabou Mohamed-Hussein[2]*, Ebtssam F. Mohammed[1], Omnia Abd El-Moneum Mohamed[1], Hanan O Mohamed[1] and Manal Mohamed Tammam Mahmoud[1]**

*[1]Department of Clinical Pathology, Faculty of Medicine, Assiut University Hospitals, Assiut, Egypt*
*[2]Department of Pulmonology, Chest Department, Faculty of Medicine, Assiut University Hospitals, Assiut, Egypt*

## Abstract

**Rationale:** Airway remodelling is clinically defined as persistent airflow obstruction despite aggressive anti-inflammatory therapies. Assessment of airway remodelling by analysis of serum markers has been developed as minimally invasive procedures.

**Aim of the work:** Evaluate the usefulness of estimation of serum MMP-9, TIMP-1 and their ratio, as non-invasive markers of airway remodelling in asthmatics.

**Patients and methods:** The study included 68 asthmatics and 20 controls. Patients were classified according to levels of asthma control, severity and disease duration. Serum samples were taken to estimate levels of MMP-9, TIMP-1 and their ratio.

**Results**: Serum MMP-9, TIMP-1, and MMP-9/TIMP-1 were significantly higher in asthmatics than controls, in uncontrolled vs. controlled asthma (P<0.001). Also they were significantly higher in severe and moderate asthma than in mild asthma (P<0.000), and in patients with disease duration >5 years vs. <5 years (P<0.001). There was significant positive correlation between studied parameters (p<0.000) and significant negative correlation between the biomarkers and $FEV_1$ (p<0.001).

**Conclusion**: Serum MMP-9, TIMP-1, and MMP-9/TIMP-1 could be considered as non-invasive markers of airway remodelling that can bypass biopsy sampling. Serum sample is easily handled and not subjected to technical error as sputum or BAL fluid.

**Keywords:** Asthma; Remodelling; MMP-9; TIMP-1; Biomarkers

## Introduction

Airway remodelling encompasses the structural alterations in asthmatic compared with normal airways. Airway remodelling in asthmatic patients involves a wide array of pathophysiologic features, including epithelial changes, increased smooth muscle mass, increased numbers of activated fibroblasts, myofibroblasts, sub epithelial fibrosis, and vascular changes. Multiple cytokines, chemokines, and growth factors released from both inflammatory and structural cells in the airway tissue create a complex signalling environment that drives these structural changes [1,2]. However, investigations have changed our understanding of asthma from a purely inflammatory disease to a disease in which both inflammatory and structural components are equally involved [1].

Matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs) are thought to contribute to the pathogenesis of asthma via their influence on the function and migration of inflammatory cells as well as matrix deposition and degradation. TIMPs inactivate MMPs by binding them in a 1:1 ratio. Thus, an increase in the molar ratio of MMP/TIMP may favour tissue injury, while the reverse could be associated with increased fibrosis [3,4]. It is possible that the increased levels of TIMP-1 observed in asthmatics may represent an endogenous protective mechanism to down-regulate the proteolytic activity of MMPs in lung parenchyma or that an excess of TIMP-1 could lead to airway fibrosis [5]. MMP-9 is the predominant MMP in asthma, and its expression is enhanced when patients have spontaneous exacerbations or in response to local instillation of allergen in the airway. As acute inflammation resolves, MMP-9 levels return toward normal [4,5].

## Aim of the Work

1. Evaluate the usefulness of serum MMP-9 and TIMP-1 determination as non-invasive markers of airway remodelling in asthmatic patients.
2. Evaluate the association of these parameters with asthma control, severity and disease duration.

## Patients and Methods

The study included 68 adult asthmatic patients attending Assiut University Hospital, Chest outpatient Clinic, their ages ranged from (18-64 years) and 20 apparently healthy subjects, age and sex matched as control group. Patients were diagnosed as bronchial asthma according to the Global Initiative for Asthma (GINA) criteria and also they were classified according to different levels of asthma control in the last three months [6] into: controlled asthma: 29 patients, and uncontrolled asthma: 39 patients. According to asthma severity the patients were divided into: mild: 14 patients, moderate 25 patients and severe: 29

***Corresponding author**: Aliaë AR Mohamed-Hussein, MD, Professor of Pulmonology, Faculty of Medicine, Assiut University, Egypt, Tel:+201222302352; E-mail: massah_99@yahoo.com

**Received** April 18, 2015; **Accepted** June 01, 2015; **Published** June 04, 2015

**Citation:** El-Moneum Hassan NA, Mohamed-Hussein AAT, Mohammed EF, El-Moneum Mohamed OA, Mohamed HO, et al. (2015) Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) as Non-Invasive Biomarkers of Remodelling in Asthma. J Pulm Respir Med 5: 266. doi:10.4172/2161-105X.1000266

patients, and according to disease duration into less than 5 years: 34 patients and more than 5 years: 34 patients.

The following are considered as exclusion criteria: Postoperative period, acute coronary artery disease, collagen diseases, cancers, chronic renal and hepatic diseases, Diabetes mellitus, collagen vascular diseases, pregnancy and smoking. Patients who received any systemic corticosteroid therapy in the last three months were also excluded.

### All patients and controls included in this study were subjected to the following

- Careful history taking and clinical examination.
- Chest X-Ray and pulmonary function tests {as Forced vital capacity (FVC), Forced expiratory volume in the first second ($FEV_1$) and $FEV_1$/FVC ratio} were done utilizing Sensor Medics Corporation Spirometer (Model CA92687, SN 54065, Osaka, Japan).
- Blood samples were taken and serum was separated for all patients and controls and subjected to the following investigations:

  i) **Routine investigations:** Complete blood picture on micros 60, Serum glucose level, liver function test, renal function test on Hitachi 911-Boehringer Mannheim.

  ii) **Special investigations:** Serum levels of MMP-9 and TIMP-1 were determined by sandwich enzyme-linked immunosorbent assay (ELISA) by using R&D system quantikine kit catalog no. DMP 900. And DTM 100 respectively, purchased from R & D system USA.

The study was approved by Faculty of Medicine, Assiut University Ethical Committee and all participants gave informed signed consent.

### Statistical analysis

Data entry and analysis are done by using SPSS software v.17 (Chicago USA). Continuous values were described by mean and standard deviation. Univariate analysis for determining the difference of lab variables between studied groups was performed using student's T test for parametric variables. The Analysis of Variance (ANOVA) was used in determining the difference of lab variables in cases where there are more than two groups. Correlations among the studied variables were tested by spearman's correlation coefficient. Difference was statistically significant if P value was less than 0.05.

## Results

Table 1 shows that serum levels of MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio in asthmatics (1181 ± 394, 569 ± 126 and 2.04 ± 0.32 respectively) were significantly higher than in controls (538 ± 155, 345 ± 71 and Ratio=1.56 ± 0.35), P<0.001. Also, uncontrolled asthmatics recorded significantly higher serum levels of MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio (1425 ± 309, 635 ± 88, Ratio 2.23 ± 0.28) compared to controlled asthma (853 ± 219, 480 ± 114, Ratio 1.78 ± 0.15 respectively), P < 0.001 (Table 2). In Table 3 it was demonstrated that serum level of MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio in patients with severe asthma (1323 ± 367 ng/ml, 643 ± 81, Ratio 2.15 ± 0.32) were significantly higher than in patients with moderate asthma (1229 ± 330ng/ml, 568 ± 107 ng/ml, Ratio 2.03 ± 0.32) (P<0.001, P<0.001, and P<0.027 respectively) and both severe and moderate asthmatics have significantly higher levels than mild asthma (800 ± 318 ng/ml, 418 ± 97 ng/ml, Ratio 1.86 ± 0.28) (P<0.001 each).

As regards disease duration, serum levels of MMP-9, and TIMP-1 in patients with disease duration (>5yrs) was (1346 ± 363 ng/ml, 641 ± 82 ng/ml) and significantly higher than in patients with disease duration (<5yrs) (1016 ± 356 ng/ml, 497 ± 121) (P<0.001 for each) (Table 4).

There was a negative correlation between FEV1% with MMP-9, and TIMP-1 (r=-0.604 p<0.001 and r=-0.742 p<0.001 respectively) and positive correlation between MMP-9 with TIMP-1 (r=0.934 p<0.001) (Figure 1A-1C).

## Discussion

Airway remodelling is clinically defined as persistent airflow obstruction despite aggressive anti-inflammatory therapies. It also leads to a decrease in lung function and airway hyperresponsiveness [1,2].

| Items | Control group (n=20) | Asthma patients (n=68) | P-value |
|---|---|---|---|
| MMP-9 (ng/ml) | | | <0.001 |
| Range | 320-890 | 550-2000 | |
| Mean ± SD | 538 ± 155 | 1181 ± 394 | |
| TIMP-1 (ng/ml) | | | <0.001 |
| Range | 210-470 | 320-810 | |
| Mean ± SD | 345 ± 71 | 569 ± 126 | |
| MMP-9/TIMP-1 Ratio | | | <0.001 |
| Range | 1.1-2.3 | 1.5-2.6 | |
| Mean ± SD | 1.56 ± 0.35 | 2.04 ± 0.32 | |

**Table 1:** Serum levels of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and MMP-9/TIMP-1 ratio in control and asthma groups.

| Items | Controlled asthma (n=29) | Uncontrolled asthma (n=39) | P-value |
|---|---|---|---|
| MMP-9 (ng/ml) | | | <0.001 |
| Range | 550-1190 | 900-2000 | |
| Mean ± SD | 853 ± 219 | 1425 ± 309 | |
| TIMP-1 (ng/ml) | | | <0.001 |
| Range | 320-670 | 450-810 | |
| Mean ± SD | 480 ± 114 | 635 ± 88 | |
| MMP-9/TIMP-1 Ratio | | | <0.001 |
| Range | 1.5-2.2 | 1.6-2.6 | |
| Mean ± SD | 1.78 ± 0.15 | 2.23 ± 0.28 | |

**Table 2:** Serum levels of MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio in patients with controlled and uncontrolled asthma. Independent samples t-test.

| Items | (Asthma severity) | | | P-value |
|---|---|---|---|---|
| | Mild (n=14) | Moderate (n=25) | Severe (n=29) | |
| MMP-9 (ng/ml) | | | | P1-2 <0.001 |
| Range | 570-1450 | 550-1970 | 870-2000 | |
| Mean ± SD | 800 ± 318 | 1229 ± 330 | 1323 ± 367 | |
| TIMP-1 (ng/ml) | | | | P1-2 <0.001 |
| Range | 320-610 | 320-750 | 530-810 | |
| Mean ± SD | 418 ± 97 | 568 ± 107 | 643 ± 81 | |
| MMP-9/TIMP-1 Ratio | | | | P1 0.027 P2 NS |
| Range | 1.51-2.4 | 1.55-2.53 | 1.57-2.64 | |
| Mean ± SD | 1.86 ± 0.28 | 2.03 ± 0.32 | 2.15 ± 0.32 | |

P1=mild vs. moderate asthma.
P2=moderate vs. severe asthma.

**Table 3:** Serum levels of MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio in different asthma severity. ANOVA test.

| Items | Duration | | P-value |
|---|---|---|---|
| | <5 years (n=34) | >5 years (n=34) | |
| MMP-9 (ng/ml) | | | <0.001 |
| Range | 550-1750 | 870-2000 | |
| Mean ± SD | 1016 ± 356 | 1346 ± 363 | |
| TIMP-1 (ng/ml) | | | <0.001 |
| Range | 320-720 | 510-810 | |
| Mean ± SD | 497 ± 121 | 641 ± 82 | |
| MMP-9/TIMP-1 Ratio | | | 0.400 |
| Range | 1.51-2.64 | 1.55-2.63 | |
| Mean ± SD | 2.00 ± 0.32 | 2.07 ± 0.33 | |

**Table 4:** Serum levels of MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio in patients with asthma duration less than 5 years (<5yrs.) and more than 5 years (>5yrs.) groups. Independent samples t-test.

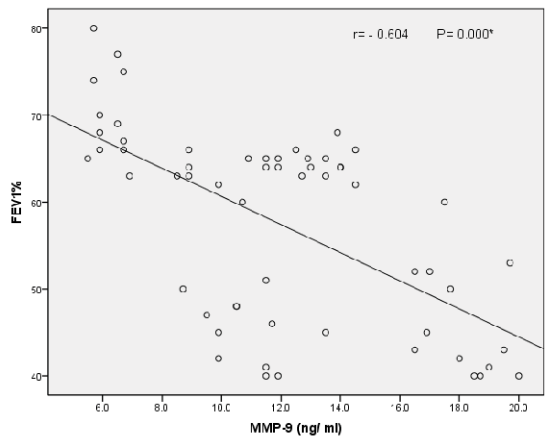


**Figure 1A:** Negative correlation between (FEV1%) and MMP-9. Correlation between Forced expiratory volume 1(FEV1) and Matrix metalloproteinase-9(MMP-9).

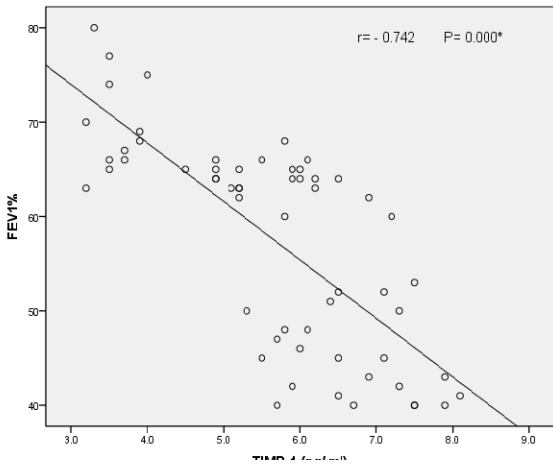


**Figure 1B:** Negative correlation between FEV1% and TIMP-1. FEV1 and Tissue inhibitor metalloproteinase-1(TIMP-1).

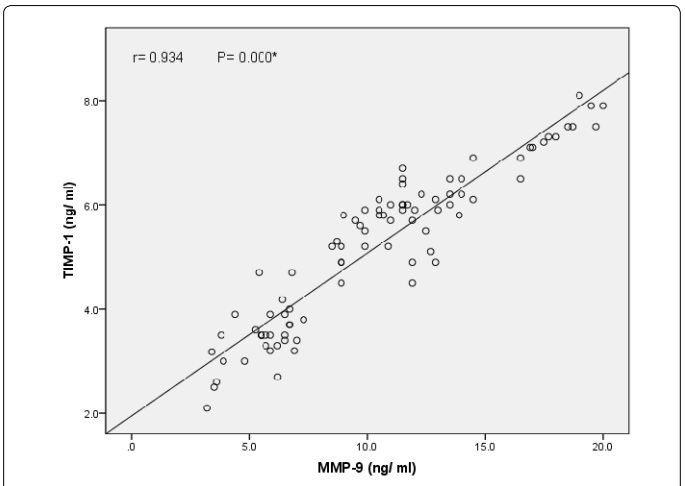


**Figure 1C:** Positive correlation between MMP-9 and TIMP-1.Correlation between MMP-9 and TIMP-1.

The current study revealed that the serum levels of MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio were significantly higher in asthmatics compared to that of controls. In asthma, the potentially most important members of remodelling markers are MMP-9 and TIMP-1. They contribute to altered extracellular matrix (ECM) turnover, influx of pro-inflammatory cells, epithelial repair, and angiogenesis [6,7]. Basement membrane degeneration by MMP-9 and other proteinases may occur in the early phase of asthma exacerbation [8]. Overexpression of TIMP-1 causes deposition of extra-cellular matrix (ECM) and thickening of basement membrane by inhibiting degradation of ECM [9].

Many authors reported that serum levels of MMP-9 were increased in asthma compared with those seen in healthy control subjects [5,10,11]. Others reported that serum MMP-9 levels of asthmatic children were significantly higher than those of healthy controls [2,12,13]. These findings suggest that an increase in the serum MMP-9 level may indicate a defect in ECM homeostasis even in stable asthmatic children and perhaps propose MMP-9 as a non-invasive marker of inflammation and remodelling in asthma. A slightly increased TIMP-1 level may indicate homeostasis of a repair process [12].

Moreover, it was found that MMP-9 and TIMP-1 concentrations as well as MMP-9/TIMP-1 ratio, were increased in the sputum of asthma and chronic bronchitis patients, and are thought to be related to airflow obstruction [5,14,15]. Karakoc and coworkers reported that children with asthma showed significantly higher MMP-9 levels of EBC (Exhaled breath condensate) in comparison with the controls [16]. The levels of MMP-9 and TIMP-1 in BALF were higher in asthma patients than in participants without asthma [17].

The MMP-9 levels were increased after allergen challenge in the

sputum from allergic asthmatics as compared to control subjects. This observation suggests that extracellular matrix can be aggressed after allergen contact and are linked to allergen-induced acute bronchial inflammation [18,19]. However, others reported that TIMP-1 levels did not vary significantly compared to controls [15,20].

The results of the current study revealed that the serum levels of MMP-9, TIMP-1, and MMP-9 /TIMP-1 ratio were significantly higher in patients with uncontrolled asthma than in patients with controlled asthma. It was reported that serum MMP-9 level was increased in asthma exacerbation and after allergenic challenge and decreased by corticosteroids [4,5]. The circulating and sputum MMP-9 concentrations were increased in patients with asthma exacerbations compared with patients with stable asthma. This increased activity may be related to exaggerated airway inflammation and airway remodelling. Circulating MMP-9 levels may therefore reflect a "spill over" of MMP-9 produced in the airways. In this context, MMP-9 may be a potential target for the management of exacerbations of asthma. In contrast, Oshita and his group reported that there were no significant differences in the TIMP-1 concentrations, suggesting that MMP-9 plays the main role in the pathophysiology of asthma exacerbations [21].

The imbalance between MMPs and TIMPs is present in acute asthma or following allergen challenge with an excess of MMP-9. This causes a high ratio of MMP-9/TIMP-1 during exacerbation and before start of treatment. The on-going airway tissue destruction and, over time with corticosteroid treatment, the TIMP-1 levels may rise, and results in dropping of the ratio of MMP-9/TIMP-1. It was suggested that overproduction of MMP-9 and TIMP-1 in acute asthma might contribute to airway tissue remodelling and that TIMP-1 production might not be suppressed by glucocorticosteroids [22-24]. It was found that Serum MMP-9 and its ratio levels were inversely correlated with airway wall thickness measured by HRCT even after correction to body surface area (WA%, WA/$\sqrt{}$BSA), and TIMP-1 levels were positively correlated with wall area and thickness (WA/BSA and T/BSA) [24]. This emphasise the need of new therapeutic modalities to avoid and reverse these structural changes.

This study revealed MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio were significantly higher in cases with severe and moderate disease than in cases with mild disease. This is similar to many authors who reported that serum levels of MMP-9 and TIMP-1 increased significantly with increasing disease severity in asthmatic patients and that MMP-9 serum levels of severe asthmatics were also increased when compared to milder asthmatics [21,25,26]. They added that MMP-9 activity is increased in the bronchial biopsy specimens, blood, induced sputum, and BAL fluid (BALF) of patients who have severe or uncontrolled asthma, severity of asthma is also a factor influencing protease secretion in airways [20,21,27].

The MMP-9 levels in sputum from patients with severe asthma, particularly those with irreversible airway obstruction, were even higher when compared with either healthy subjects or patients with mild asthma. However no significant difference was seen between normal subjects and patients with mild asthma. Furthermore, MMP-9/TIMP-1 ratio in sputum of acute severe asthmatics was higher than in the other groups. These findings may indirectly indicate the involvement of MMP-9 in airway remodelling process in patients with severe asthma who have an irreversible component of airway narrowing. Therefore, MMP-9 level measurements could be proposed as a useful tool to determine one of the aspects of disease severity [18,20].

Concerning the relation between duration of asthma and the studied biomarkers, the results of this study revealed that serum MMP-9 and TIMP-1 were significantly higher in patients with disease duration >5 years than in those with disease duration <5 years. It is generally accepted that chronic inflammation leads to airway remodelling in patients with asthma and that this may contribute to irreversible airflow obstruction in affected individuals [21,28]. In a study by Chung and Kimb [29] they found that serum MMP-9 in children with persistent asthma was significantly greater than in children who were diagnosed with asthma for the first time [29]. It was reported that the duration of asthma has been associated with reduced lung function, increased airway hyper-responsiveness (AHR) and asthma symptoms, as well as greater use of medications. The remodelling process has been proposed to explain these features. The Childhood Asthma Management Program (CAMP) study demonstrated an association between asthma duration and reduced lung function, higher AHR; greater asthma symptomatology. Bergeron and colleagues suggested that this may be related to airway remodelling [30].

To study the correlation between performed pulmonary functions and the studied parameters, the results revealed that there were significant negative correlations between MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio with pulmonary function tests FVC%, FEV1% and FEV1/FVC%.

In agreement, authors reported a significant negative correlation between serum MMP-9 levels and both the maximum per cent fall in forced expiratory volume at the first second ($FEV_1$%) during the late response [5]. Others showed that elevated sputum MMP-9 level were associated with a fall in $FEV_1$ after allergen challenge and were linked to asthma severity [31,32]. Sputum levels of MMP-9 measured after bronchial challenge with common allergens were significantly positively correlated with the maximal fall in FEV-1 observed during the asthmatic reaction [4]. Barbaro and colleagues in 2014 reported a positive correlation between MMP-9 level in exhaled breath and the percentage of $FEV_1$ in severe asthmatics [9] and serum TIMP-1 concentration was negatively correlated with the $FEV_1$/FVC% in asthmatic patients [14]. The level of $FEV_1$ was positively correlated with the serum MMP-9: TIMP-1 ratio, but weakly positively correlated with the MMP-9 level [33].

Taken together, the evaluation of matrix metalloproteases and of their inhibitors is certainly indirect, but may be useful as markers of airway inflammation and remodelling. Further studies are needed to measure the impact of new anti-asthma drugs on these markers and on asthma control.

## Conclusion

Serum levels of MMP-9, TIMP-1 and MMP-9/TIMP-1 are increased in asthmatic patients and are associated with increased asthma severity, asthma exacerbation and prolonged disease duration and negatively correlate with pulmonary function tests. Serum MMP-9, TIMP-1 and MMP-9/TIMP-1 could be considered as non-invasive markers of airway remodelling that can bypass biopsy sampling. The serum sample is easily handled and not subjected to technical error as sputum or bronchoalveolar lavage fluid.

### References

1. Al-Muhsen S, Johnson JR, Hamid Q (2011) Remodeling in asthma. J Allergy Clin Immunol 128: 451-462.

2. Zhang YY, Xu HZ, Bao XE (2014) Detection and Clinical Significance of a Potential Mediator of Airway Remodeling in Preschool Wheezy Children HK J Paediatr (New Series) 19: 63-70.

3. Chaudhuri R, McSharry C, Brady J, Donnelly I, Grierson C, et al. (2012) Sputum matrix metalloproteinase-12 in patients with chronic obstructive pulmonary

disease and asthma: relationship to disease severity. J Allergy Clin Immunol 129: 655-663.

4. Castano R, Miedinger D, Maghni K, Ghezzo H, Trudeau C, et al. (2013) Matrix metalloproteinase-9 increases in the sputum from allergic occupational asthma patients after specific inhalation challenge. Int Arch Allergy Immunol 160: 161-164.

5. Mohamed GM, Nazmy Farres M, Mahmoud H (2012) Interplay between matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in acute asthma exacerbation and airway remodeling. Egyptian Journal of Chest Diseases and Tuberculosis 61: 35-39.

6. Polosa R, Bellinvia S, Caruso M, Emma R, Alamo A, et al. (2014) Weekly low-dose methotrexate for reduction of Global Initiative for Asthma Step 5 treatment in severe refractory asthma: study protocol for a randomized controlled trial. Trials 15: 492.

7. Koloze MT (2010) The Role Of Matrix Metalloproteinases (MMPs) And Their Proteolytic Degradation Of Chemokines In The Lung Submitted In Partial Fulfillment Of The Requirements For The Degree Of Master of Science Thesis Advisor: Dr. Tracey L. Bonfield Department Of Pathology Case Western Reserve University May, 2010.

8. Vignola AM, Mirabella F, Costanzo G, Di Giorgi R, Gjomarkaj M, et al. (2003) Airway remodeling in asthma. Chest 123: 417S-22S.

9. Barbaro MP, Spanevello A, Palladino GP, Salerno FG, Lacedonia D, et al. (2014) Exhaled matrix metalloproteinase-9 (MMP-9) in different biological phenotypes of asthma. European Journal of Internal Medicine 2: 92-96.

10. Cataldo D, Munaut C, Noël A, Frankenne F, Bartsch P, et al. (2000) MMP-2- and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. Int Arch Allergy Immunol 123: 259-267.

11. Simpson JL, Scott RJ, Boyle MJ, Gibson PG (2005) Differential proteolytic enzyme activity in eosinophilic and neutrophilic asthma. Am J Respir Crit Care Med 172: 559-565.

12. Dogu F, Yildiran A, Loglu D (2008) Serum Transforming Growth Factor-ß 1(TGF-ß1),Matrix Metalloproteinase-2 (MMP-2),Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitors of Metalloproteinase (TIMP-1) Levels in Childhood Asthma. Turk J Med Sci 38: 415-419.

13. Hong Z, Lin YM, Qin X, Peng JL (2012) Serum MMP-9 is elevated in children with asthma. Mol Med Rep 5: 462-464.

14. Higashimoto Y, Yamagata Y, Iwata T, Okada M, Ishiguchi T, et al. (2005) Increased serum concentrations of tissue inhibitor of metalloproteinase-1 in COPD patients. Eur Respir J 25: 885-890.

15. Boulay ME, Prince P, Deschesnes F, Chakir J, Boulet LP (2004) Metalloproteinase-9 in induced sputum correlates with the severity of the late allergen-induced asthmatic response. Respiration 71: 216-224.

16. Karakoc GB, Yukselen A, Yilmaz M, Altintas DU, Kendirli SG (2012) Exhaled breath condensate MMP-9 level and its relationship with asthma severity and interleukin-4/10 levels in children. Ann Allergy Asthma Immunol 108: 300-304.

17. Kim JS, Kang JY, Ha JH, Lee HY, Kim SJ, et al. (2013) Expression of nerve growth factor and matrix metallopeptidase-9/tissue inhibitor of metalloproteinase-1 in asthmatic patients. J Asthma 50: 712-717.

18. Gueders MM, Foidart JM, Noel A (2006) Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: Potential implications in asthma and other lung diseases. European Journal of Pharmacology 533: 133-144.

19. Felsen CN, Savariar EN, Whitney M, Tsien RY (2014) Detection and monitoring of localized matrix metalloproteinase upregulation in a murine model of asthma. Am J Physiol Lung Cell Mol Physiol 306: L764-774.

20. Mattos W, Lim S, Russell R, Jatakanon A, Chung KF, et al. (2002) Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. Chest 122: 1543-1552.

21. Oshita Y, Koga T, Kamimura T, Matsuo K, Rikimaru T, et al. (2003) Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activity in exacerbations of asthma. Thorax 58: 757-760.

22. Tanaka H, Miyazaki N, Oashi K, Tanaka S, Ohmichi M, et al. (2000) Sputum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio in acute asthma. J Allergy Clin Immunol 105: 900-905.

23. Kelly EA, Jarjour NN (2003) Role of matrix metalloproteinases in asthma. Curr Opin Pulm Med 9: 28-33.

24. Mohamed-Hussein AAR, Abdel-Aziz S (2012) Imbalance between MMP-9 and its inhibitor is associated with increased airway wall thickness in uncontrolled asthmatics, Egy J Bronchol 6: 31-35.

25. Dar KA, Shahid M, Mubeen A, Bhargava R, Ahmad Z, et al. (2012) The role of noninvasive methods in assessing airway inflammation and structural changes in asthma and COPD. Monaldi Arch Chest Dis 77: 8-18.

26. Belleguic C, Corbel M, Germain N, Léna H, Boichot E, et al. (2002) Increased release of matrix metalloproteinase-9 in the plasma of acute severe asthmatic patients. Clin Exp Allergy 32: 217-223.

27. Ventura I, Vega A, Chacón P, Chamorro C, Aroca R, et al. (2014) Neutrophils from allergic asthmatic patients produce and release metalloproteinase-9 upon direct exposure to allergens. Allergy 69: 898-905.

28. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM (2000) Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 161: 1720-1745.

29. Chung H, Kimb S (2004) Increased release of matrix metalloproteinase -9 and transforming growth factor-ß1 in the plasma of children with persistent asthma-Journal of Allergy and Clinical Immunology 113: S195.

30. Bergeron C, Tulic MK, Hamid Q (2010) Airway remodelling in asthma: from benchside to clinical practice. Can Respir J 17: e85-93.

31. Cataldo DD, Bettiol J, Noel A, Bartsch P, Foidart JM, et al. (2002) Matrix metalloproteinase-9, but not tissue inhibitor of matrix metalloproteinase-1, increases in the sputum from allergic asthmatic patients after allergen challenge. Chest 122: 1553-1559.

32. Wenzel SE, Balzar S, Cundall M (2003) Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. J Allergy Clin Immunol 111: 1345-1352.

33. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M (2003) Airway structural alterations selectively associated with severe asthma. Am J Respir Crit Care Med 167: 1360-1368.