ORIGINAL ARTICLE

Serum levels of matrix metalloproteinase-9, tissue inhibitors of metalloproteinase-1 and their ratio are associated with impaired lung function in the elderly: A population-based study

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ABSTRACT

Background and objective: Matrix metalloproteinases (MMP) and their inhibitors, tissue inhibitors of metalloproteinases (TIMP), regulate homeostasis and turnover of the extra cellular matrix. The aim of this study was to investigate the associations of serum MMP-9 and TIMP-1 with lung function.

Methods: Spirometry was performed in a population-based sample of 888 subjects aged 70 years. Serum MMP-9 and TIMP-1 concentrations were measured by ELISA.

Results: Lower FEV1 values were associated with higher serum levels of MMP-9 (P = 0.001) and TIMP-1 (P < 0.001), and a higher ratio of MMP-9 to TIMP-1 (P = 0.02). These associations were significant after adjustment for gender, weight, height, BMI, current smoking, pack years of smoking and the time for which samples were frozen. After stratification for gender, the associations between FEV1 and MMP-9, TIMP-1, and their ratio, were significant in men but not in women.

Conclusions: Lower FEV1 was significantly but weakly associated with higher serum levels of MMP-9, TIMP-1 and a higher MMP-9/TIMP-1 ratio. This association was stronger in men than in women, suggesting a possible role for extracellular matrix remodelling in the development of impaired lung function. These associations may also partly explain the association between low FEV1, and cardiovascular disease.

Key words: age, gender, lung function, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1.

INTRODUCTION

Matrix metalloproteinases (MMP) are crucial for the homeostasis and turnover of extracellular matrix in health and disease. The protease–antiprotease hypothesis states that an imbalance between proteolytic enzymes and their inhibitors could result in tissue proteolysis, which may be of importance in the development of impaired lung function.1 Matrix metalloproteinases are a family of proteolytic enzymes that degrade all types of extracellular matrix components.2,3 MMP-9 is a major elastolytic enzyme and is produced by a range of stromal cells, as well as alveolar macrophages and neutrophils, which play a major role in lung diseases such as COPD.4,6 MMP activity is regulated by proteolytic activation of the inactive proenzymes and through inhibition of the active enzyme by tissue inhibitors of metalloproteinases (TIMP). TIMP-1 binds to both the active and precursor forms of MMP-9, TIMP-1 and a higher MMP-9/TIMP-1 ratio. This association was stronger in men than in women, suggesting a possible role for extracellular matrix remodelling in the development of impaired lung function.1
other studies have shown that FEV₁ correlated positively with the MMP-9/TIMP-1 ratio. However, these were all relatively small, case–control studies that were not population-based.

We hypothesized that MMP may have an important role in the pathogenesis of impaired lung function, and that the extracellular matrix remodelling process may be reflected in the relationship of serum MMP-9 and TIMP-1 levels to lung function at the population level. Therefore, the relationship of serum MMP-9 and TIMP-1 levels to lung function was investigated in a large elderly population.

METHODS

Study population

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS; see http://www.medsci.uu.se/pivus/pivus.htm) study was conducted between 2001 and 2004. The design of the PIVUS study has been published in detail. All subjects aged 70 years and living in the municipality of Uppsala, Sweden, were eligible to participate in the study. Participants were chosen from the civil registry, and between April 2001 and June 2004, eligible subjects (n = 2025) were randomly invited to participate in the study, through postal invitation within 2 months of their 70th birthday. A total of 1016 subjects agreed to participate. As the participation rate was only 50.1%, cardiovascular disorders and medications were evaluated for 100 consecutive non-participants. The prevalence of cardiovascular drug use, history of myocardial infarction, coronary revascularization, use of antihypertensive medications, statins and insulin treatment were similar to those in the participants; however, the prevalence of diabetes, congestive heart failure and stroke was higher among non-participants.

The study was approved by the Ethics Committee at the University of Uppsala, and all participants gave informed consent.

Clinical investigations

Participants completed a questionnaire concerning their medical history, regular medication use and smoking habits. All subjects were examined the morning after an overnight fast: no medication or smoking was permitted after midnight before the examination. Height, weight and abdominal and hip dimensions were recorded, and blood samples were taken for analysis by standard laboratory techniques. BMI was calculated as weight in kilograms divided by the square of height in metres. Subjects were categorized as never-smokers, ex-smokers or current smokers. Pack-years of smoking were calculated from the available data on number of cigarettes smoked per week and the duration of smoking in years.

Lung function tests

The spirometric evaluations performed in the PIVUS study have been described previously. FEV₁ was measured with a Vitalograph Alpha spirometer (Vitalograph Ltd, Buckingham, England), in accordance with the American Thoracic Society recommendations. The best of three values recorded was used in the analysis. FEV₁ values were expressed as percent of predicted values, adjusted for age, gender and height. Predicted values for FEV₁ were based on the European Coal and Steel Union reference values.

Serum matrix metalloproteinase-9 and tissue inhibitors of metalloproteinase-1 measurements

Serum MMP-9 and TIMP-1 levels were analysed using commercial ELISA from R&D Systems Europe Ltd. (Abingdon, UK). Blood samples were placed in glass test tubes without additives, then centrifuged 1 h after sampling, and the serum frozen at −70°C until analysed. Samples were thawed for analysis an average of 776 days (range 456–1124) after sampling. Analysis of samples from 20 subjects, twice on the same plate, gave coefficients of variation of 5.4% for MMP-9 and 4.8% for TIMP-1. The ratio of MMP-9 to TIMP-1 in serum was calculated.

Statistical analysis

The distribution of MMP-9 and TIMP-1 values was examined and natural logarithmic transformation was performed in order to normalize the distributions. Simple linear regression was used to assess the association of matrix factors (serum levels of MMP-9, TIMP-1 and the MMP-9/TIMP-1 ratio) with FEV₁. The independent association between FEV₁ (expressed in millilitres) and the matrix factors were analysed by multiple linear regression with adjustment for gender, age, height, BMI, pack-years of smoking, current smoking and time for which the sample was frozen, as confounding variables. The matrix factors were standardized to one SD in all regression models. Potential interactions between the matrix factors and gender, BMI, pack-years of smoking and current smoking were investigated in all models. All models were investigated in gender-pooled and gender-specific strata, and gender was included as a covariate in all gender-pooled analyses, in addition to the covariates listed previously. The STATA 9 programme was used for all analyses and P-values < 0.05 were considered significant.

RESULTS

Characteristics of the study population

The study included 888 subjects with acceptable spirometry results and data for serum MMP-9 and
TIMP-1 levels. The characteristics of these participants are presented in Table 1.

**Characteristics of the non-participants**

The 128 subjects who did not participate in the evaluation of spirometry were more likely to be men than women (14.8% vs 10.4%, \( P = 0.04 \)) and the men had slightly higher TIMP-1 values than women (185 ng/mL, 95% CI: 177–194 vs 175 ng/mL, 95% CI: 173–177; \( P = 0.01 \)). There were no differences for BMI, MMP-9 levels or smoking history.

**Univariate associations**

There were inverse associations between FEV\(_1\)% and serum MMP-9 and TIMP-1 levels, and MMP-9/TIMP-1 ratio (Fig. 1). Higher MMP-9 and TIMP-1 levels were associated with increased BMI (\( P \)-value for trend < 0.0001), as was the MMP-9/TIMP-1 ratio (\( P \)-value for trend 0.005). MMP-9 and TIMP-1 levels were also associated with smoking status, being lowest in never-smokers and highest in current smokers.

**Multivariate regression models**

The associations between the matrix factors and FEV\(_1\) remained significant after adjustment for gender, age, height, BMI, current smoking, pack-years of smoking and the time for which the sample was frozen (Table 2). A significant gender interaction was identified in the associations between MMP-9, TIMP-1 and FEV\(_1\) (\( P = 0.002 \)), and between MMP-9/TIMP-1 ratio and FEV\(_1\) (\( P = 0.02 \)). After stratification for gender, the associations between the matrix factors and FEV\(_1\) were statistically significant for men but not for women (Table 2). There was no interaction between smoking and BMI.

**DISCUSSION**

In this population-based study, weak but significant inverse associations were identified between the matrix factors and FEV\(_1\) in elderly subjects. After stratification for gender, these associations were significant for men but not for women.

Consistent with previous studies, higher serum levels of MMP-9 were associated with lower FEV\(_1\).\(^{16,17}\) Studies in animals,\(^{18}\) and on BAL, tissue samples and sputum from humans,\(^{5,19–23}\) indicate that MMP-9 and TIMP-1 are candidate proteinases in the pathogenesis of COPD. In this study, both MMP-9 and TIMP-1 were elevated in participants with impaired lung function, suggesting an underlying proteolytic environment in subjects with poor lung function in general, and not just in subjects with COPD. Impairment of lung function correlated with MMP-9 and TIMP-1 ratio, indicating that a chronic imbalance in MMP-9 and TIMP-1 may be an important factor in the development of poor lung function in this elderly population.

Impaired lung function was inversely correlated with MMP-9, TIMP-1 and their ratio in men, whereas no such association was identified for women. A gender difference in the association of CRP levels with FEV\(_1\) decline, with a stronger inverse association between CRP and FEV\(_1\) in men than in women, has been reported.\(^{24,25}\) Furthermore, there was an association between a faster rate of decline in FEV\(_1\) and higher CRP levels in men but not in women.\(^{24}\) Hormonal changes in women affect low-grade inflammation,\(^{26}\) and an increase in inflammatory mediators, paralleling a rise in cardiovascular risk has been observed after menopause.\(^{27}\) A large retrospective study of 1053 patients with severe emphysema revealed gender differences, with women having more dyspnoea, anatomically smaller airway lumens, disproportionately thicker airway walls and less extensive emphysema, characterized by smaller emphysematous lesions and less peripheral involvement, compared with men.\(^{28}\) It is possible that gender-specific hormonal or anatomical differences may be related to differences in inflammatory activity, which may, in turn, influence the pathogenesis of airflow limitation.

The prevalence of smoking in the present study population was similar to that reported for other elderly populations.\(^{29}\) Increased levels of MMP-9 and TIMP-1 are associated with high BMI, and an...
imbalance in the MMP-9/TIMP-1 ratio is postulated to be a pathophysiological background for many of the diseases associated with obesity, such as cardiovascular disease. Reduced lung function is associated with cardiovascular morbidity and mortality, and even a modest decline in FEV₁ has been associated with a fivefold increase in death due to ischaemic heart disease, independently of age, gender and smoking history. Circulating MMP-9 and TIMP-1 are associated with cardiovascular diseases and may reflect extracellular matrix degradation consequent to cardiovascular remodelling. Thus, it is possible that MMP-9, TIMP-1 and the balance between these two enzymes are key to the association between impairment of lung function and cardiovascular disease.

One of the strengths of this study is that the data were collected from a general population, and assessed using high-quality, standardized methods. However, the limitations of the study merit some discussion. First, the ELISA used to measure MMP and TIMP.

![Figure 1](image-url) Correlations between FEV₁% and serum levels of matrix metalloproteinase (MMP)-9, tissue inhibitor of metalloproteinase (TIMP)-1 and the ratio of MMP-9 to TIMP-1.

**Table 2** Multivariate adjusted models for the association of MMP-9 and TIMP-1 levels with FEV₁

<table>
<thead>
<tr>
<th>Models adjusted for gender, BMI, pack years and current smoking</th>
<th>MMP-9</th>
<th>TIMP-1</th>
<th>MMP-9/TIMP-1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁, β-coefficient (95% CI) in mL per 1 SD change in matrix marker</td>
<td>All¹</td>
<td>-40 (-72, -8)</td>
<td>-44 (-76, -11)</td>
</tr>
<tr>
<td></td>
<td>Men²</td>
<td>-81 (-134, -28)</td>
<td>-55 (-104, -6)</td>
</tr>
<tr>
<td></td>
<td>Women³</td>
<td>2 (-35, 40)</td>
<td>-27 (-68, 14)</td>
</tr>
</tbody>
</table>

¹ Adjusted for gender, age, height, BMI, current smoking, pack-years and time for which the sample was frozen.
² Adjusted for age, height, BMI, current smoking, pack-years and time for which the sample was frozen.
³ MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.
TIMP in serum detect both total enzyme and pro-enzyme; ideally, a correlation should be established between tissue activity and circulating levels of MMP-9 and TIMP-1. Second, it has been suggested that MMP-9 levels do not reflect overall MMP activity in the airways, as COPD is associated with higher levels of MMP-9 but not with increased MMP activity. Third, post-bronchodilator measurements of FEV1 were not performed. Fourth, it has been suggested that MMP-9 in plasma may degrade over time when stored at ~80°C, although TIMP-1 levels appear to remain stable. The pattern and cause of this degradation is uncertain and studies on samples from this study indicated a small, but statistically significant, increase in TIMP-1 levels but no definite change in MMP-9 levels over time. Although the participation rate was only 50.1%, there were no major differences between non-participants and participants, as indicated by an analysis of a subsample of the population.

In conclusion, this study has shown that lower FEV1 is weakly associated with higher levels of MMP-9, TIMP-1 and a higher MMP-9/TIMP-1 ratio. These matrix factors account for a very small proportion of the observed variance in lung function parameters. The association was stronger in men than in women, and may indicate a role for extracellular matrix remodelling in the development of impaired lung function. These associations may also partly explain the link between low FEV1 and cardiovascular disease.

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REFERENCES

Association of MMP-9 and TIMP-1 with FEV1


