Variability in proteinase-antiproteinase balance, nutritional status, and quality of life in stable chronic obstructive pulmonary disease due to tobacco and nontobacco etiology

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ABSTRACT

Context: Although the role of proteinase/antiproteinase imbalance in chronic obstructive pulmonary disease (COPD) due to tobacco is well established, information in COPD due to nontobacco etiology is sparse. Aims: To assess the variability in metalloproteinase activity in COPD related to tobacco and nontobacco causes. Settings and Design: This is a hospital-based, prospective, observational study. Subjects and Methods: Serum matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1) were estimated in 200 subjects divided equally into four groups, i.e. COPD in tobacco smokers, COPD in nonsmokers but with exposure to biomass-related indoor air pollution, smokers without COPD, and nonsmoking healthy controls. Anthropometric skinfold measurements, quality of life (QOL) using St. George Respiratory Questionnaire, and exercise capacity using the 6-min walk test (6-MWT) were carried out. Groups were compared using analysis of variance and Kruskal–Wallis plus Mann–Whitney U-test to assess differences between groups. The Chi-square and Fisher’s exact tests were used to evaluate associations among categorical variables. Spearman’s rank correlation was calculated to assess the correlation between data. Results: Patients with COPD due to either tobacco or nontobacco etiology were older, more malnourished, had worse QOL, and poorer exercise capacity compared to non-COPD subjects. Triceps, subscapular, and suprailiac skinfold thicknesses were less in smokers with COPD than biomass-related COPD. MMP-9 and TIMP-1 levels were similar across all groups. TIMP-1 significantly correlated with 6-MWT among all groups. Conclusions: The protease-antiprotease balance in COPD is similar irrespective of the presence or absence of tobacco exposure but is related to poor exercise capacity.

KEY WORDS: Biomass, chronic obstructive pulmonary disease, matrix metalloproteinase-9, tissue inhibitor of metalloproteinases-1, tobacco

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of respiratory morbidity and mortality worldwide, and it is predicted to become the third leading cause of death and the fifth leading cause of disability by the year 2020.[1,2] Although COPD is most commonly caused by smoking, several other risk factors such as exposure to occupational...
Toxins, air pollution, passive smoking, and indoor air pollution are well known.\(^{1-4}\) Indoor air pollution, in particular, has been implicated as an important risk factor for the development of COPD, particularly in women of developing countries where the use of coal and biomass fuels (dung, crop residues, and wood) is used widely for cooking and space heating.\(^{5-7}\)

The pathogenesis of COPD is related to chronic inflammations of airways, parenchyma, and pulmonary vasculature, imbalance between proteinases and antiproteinases in the lung, and oxidative stress.\(^{8-10}\) Currently, the theory of proteinase/antiproteinase imbalance is most widely accepted as a likely factor causing emphysema.

Studies of human samples have shown an increase in many proteases, including matrix metalloproteinase (MMP) in smoking-related emphysema. Several members of the MMP family such as MMP-1, MMP-2, MMP-8, MMP-9, and MMP-12 are elevated both in experimental emphysema and human COPD.\(^{11-13}\)

It has also been observed that levels of MMPs, especially MMP-9, are elevated in the bronchoalveolar lavage (BAL) fluid from patients with COPD compared to normal controls.\(^{10,11}\) Furthermore, elevated levels of MMP-9 and its related inhibitor, tissue inhibitor of metalloproteinases-1 (TIMP-1), have been found in sputum from patients with chronic bronchitis with a correlation with declining lung function.\(^{12-14}\)

Since some of these matrix markers are easily measurable in serum or plasma, the possibility of monitoring matrix turnover by means of simple blood tests is a promising concept for understanding the pathogenesis of COPD and developing future therapeutic interventions.

While the role of inflammation and proteinase/antiproteinase balance in COPD due to tobacco smoking has been well established, the corresponding information in patients who develop COPD due to nontobacco-related factors is not so well known. In addition, there is a paucity of data regarding the role of circulating metalloproteinases in COPD patients with respect to the correlation with systemic inflammatory process in smoker and even less in COPD due to nontobacco etiology.

The aim of the present study, therefore, was to compare the proteinase-antiproteinase imbalances between patients with COPD occurring due to tobacco or nontobacco exposure and to see their association with demographic profile and various indices of disease severity.

**SUBJECTS AND METHODS**

This cross-sectional study was conducted among outpatients of the Medical and Pulmonary Medicine Departments at a tertiary referral hospital in Northern India between July 2009 and June 2012. Approval was obtained from the Institutional Ethical Committee and written informed consent from all subjects was taken.

Consecutive patients with a diagnosis of COPD based on the medical history and the results of spirometry were included in the study. COPD was defined according to the Global Initiative for Chronic Obstructive Lung Disease guidelines.\(^{15}\) The presence of chronic cough with expectoration, breathlessness, and spirometric confirmation of airflow limitation with forced expiratory volume 1 (FEV1) of <70% predicted with reversibility of <15% predicted or <200 ml after inhalation of 400 mcg of short-acting B2-agonist was taken as diagnostic criteria.

**Study design and study population**

All participants were categorized into four groups comprising fifty subjects in each. Group I - patients with COPD who were current tobacco smokers; Group II - COPD in nonsmokers but with significant exposure to other sources such as indoor air pollution from biomass fuel consumption; Group III - smokers without COPD, i.e., subjects without any respiratory symptom, no evidence of active lung disease, and having a lifetime pack-years of smoking of at least 10, along with normal spirometry or spirometry not fulfilling the diagnostic criteria for COPD; and Group IV - nonsmoker healthy controls, i.e., subjects who were lifelong nonsmokers with normal lung functions as assessed by spirometry.

Subjects with a recent history of respiratory tract infection within the last 4 weeks, COPD patients who received systemic steroids during the previous 4 weeks before entering the study, and persons suffering from any other lung disease such as lung cancer or bronchiectasis were excluded from the study.

All participants answered queries as per a structured questionnaire which included demographic details including occupation, smoking habits, current smoking status, total smoking burden calculated as pack-years for cigarettes and smoking index for bidis (a local variety of cigarette with tobacco wrapped in tendu leaves), history of exposure to environmental pollutants, indoor smoke, type of cooking fuels used, duration of disease, medication history, symptoms, and dyspnea assessment according to the Medical Research Council dyspnea scale and visual analog scale. BODE index\(^{16}\) (body mass index [BMI], airflow obstruction, dyspnea, and exercise capacity) was calculated for each patient and quality of life (QOL) was evaluated using the St. George’s Respiratory Questionnaire. Other comorbidities such as diabetes, hypertension, ischemic heart disease, and past tuberculosis were recorded.

**Laboratory parameters**

Complete hemogram, liver and renal functions tests, and electrocardiogram were performed in all subjects. Chest radiograph was performed whenever indicated. Anthropometric measurements of skinfold thicknesses
from the right side of the body were taken from (1) biceps, (2) triceps, (3) subscapular, and (4) suprailliac areas using the Harpenden skinfold calipers (British Indicators Ltd., St Albans, Herts). At these four sites, the skinfold was pinched up firmly between the thumb and forefinger and pulled away slightly from the underlying tissues before applying the calipers for the measurements. Mid-arm circumference (MAC) was measured at the midpoint of the humeral head. Six-minute walk test was performed for all subjects using the American Thoracic Society (ATS) guidelines (2002).[10]

Flow-volume spirometry was done with a pneumotachograph-based spirometer by a trained technician using the ATS guidelines.[11]

Blood sampling
Blood was collected from the subjects after overnight fasting from the antecubital vein under aseptic precautions in vacuum collection tubes containing 0.5 ml sodium citrate. Serum was separated from samples by centrifugation at 3000 rpm for 10 min at ambient temperature and stored at −20°C until analysis.

Measurement of inflammatory markers
MMP-9 and TIMP-1 were measured in the thawed serum samples using commercial ELISA kits, R and D Systems, USA. The normal range of MMP-9 and TIMP-1 was calculated as the mean in controls + 2 standard deviation (SD) and expressed in ng/ml.

Statistical analysis
All data were managed on an Excel spreadsheet and presented as mean ± SD or median (range) for continuous variables and frequencies (%) for categorical variables. Groups were compared using analysis of variance and Kruskal–Wallis plus Mann–Whitney U-test to assess differences between groups as appropriate. The Chi-square and Fisher’s exact tests were used to evaluate associations among categorical variables. Spearman’s rank correlation was calculated to assess the correlation between data. In all tests, values of P < 0.05 were considered statistically significant between the groups. All statistical analyses were performed using STATA version 10.1. StataCorp. 2007., TX: StataCorp LP

RESULTS
The subjects were categorized into four groups of fifty participants each. Of the total study groups of 200 subjects, 149 (74.5%) were males. There was a male predominance in all groups, except Group IV which had equal proportion of males and females [Table 1].

The subjects of Group IV (healthy controls) were significantly younger than the other three groups. Smoking index (pack-year) was higher in Group I than Group III. Patients in Group I had lower BMI, worse anthropometric parameters (viz., biceps, triceps, subscapular, suprailliac, and MAC), and higher BODE index compared to the other three groups [Table 1]. Similarly, pulmonary functions were significantly worse in COPD patients (Groups I and II) compared to Groups III and IV although there was no statistically significant difference between Groups I and II [Table 2]. The 6-min walk distance (6-MWD) was comparable between Groups I and II but significantly lower compared to Groups III and IV [Table 2].

QOL scores of all domains were similar in Groups I and II but worse than Groups III or IV [Table 2]. However, QOL was similar between Groups I and II but not statistically different [Table 2]. The serum concentration of MMP-9 and TIMP-1 was significantly lower compared to Groups III and IV [Table 2].

Table 1: Baseline characteristics of the study subjects

| Variable                        | Group I (n=50) | Group II (n=50) | Group III (n=50) | Group IV (n=50) | P value |
|---------------------------------|---------------|----------------|-----------------|----------------|---------|
| Age (years)                     | 57±6.6        | 57.5±9.6       | 51.7±10.4       | 49.9±9.6       | 0.0001  |
| Sex distribution                |               |                |                 |                |         |
| Male                            | 46 (30.9%)    | 33 (22.1%)     | 45 (30.2%)      | 25 (16.8%)     |         |
| Female                          | 4 (7.9%)      | 17 (33.3%)     | 5 (9.8%)        | 25 (49.0%)     |         |
| Smoking index                   | 550 (50-1600) | 0              | 275 (12-1320)   | 0              | <0.01   |
| COPD duration                   | 6.4±6.1       | 5.4±4.1        | 0               | 0              | 0.78    |
| Inhaled steroid use             | 12 (24%)      | 16 (32%)       | 0               | 0              | <0.01   |
| BMI (kg/m²)                     | 19.9±3.9      | 22.4±4.6       | 22.9±3.9        | 24.6±4.1       | <0.01   |
| Biceps (mm)                     | 4.8±2.5       | 6.2±3.3        | 5.7±2.7         | 9.2±5.0        | <0.01   |
| Triceps (mm)                    | 10.4±5.3      | 14.4±6.2       | 11.5±5.4        | 17.2±6.7       | <0.01   |
| Subscapular thickness (mm)      | 11.8±5.4      | 15.3±7.7       | 14.3±5.5        | 21.2±7.8       | <0.01   |
| Suprailliac thickness (mm)      | 8.9±5.0       | 12.6±7.6       | 10.7±4.1        | 16.7±6.9       | <0.01   |
| Mid arm circumference (MAC) (cm)| 3.3±0.7       | 1.9±0.7        | 3.9±0.9         | 2.8±0.8        | <0.01   |
| FEV1 (L)                        | 1.4±0.9       | 1.1±0.5        | 2.5±0.7         | 2.2±0.7        | <0.01   |
| FEV1/FVC                        | 53.6±11.1     | 58.9±10.6      | 78.6±12.8       | 78.9±8.3       | <0.01   |
| 6MWT (metres)                   | 309±138.1     | 293.1±85.9     | 392.7±107.7     | 370.4±102.7    | <0.01   |
| MMP-9 (ng/ml)                   | 440 (0-2230)  | 440.5 (0-1926) | 324 (0-1246)    | 378.5 (34-2088)| 0.14    |
| TIMP-1 (ng/ml)                  | 207.2 (79-673.5) | 220.4 (82-538.7) | 153.1 (74-792.9) | 201 (37.1-376.1)| 0.08    |
| BODE index                      | 4.7±2.3       | 3.9±2.1        | 1.1±1.3         | 0.9±1.1        | <0.01   |
| SGRO score                      | 51.1±18.7     | 51.5±16.7      | 14.7±18.0       | 12.1±18.2      | <0.01   |

All value expressed as mean ± S.D except sex distribution (number (%)) and smoking index, MMP-9, TIMP-1 (Median (min-max)); P value < 0.05 taken as significant
MMP-9 was virtually identical in Groups I and II and higher (nonsignificant) than Groups III and IV. TIMP-1 levels were also increased in serum of patients of Groups I and II compared to Group III; the difference was, however, not statistically significant.

The correlation between MMP-9 and TIMP-1 with various variables is depicted in Tables 3 and 4, respectively. 6-MWD was the only parameter which correlated significantly with MMP-9 as well as TIMP-1 across all four groups. No other consistent associations were noted between MMP-9 and TIMP-1 with other variables.

**DISCUSSION**

The chronic inflammatory response in COPD is associated with an imbalance between proteases and antiproteases.

**Table 2: Within group comparisons of various parameters between four groups**

| Variable | I/II | I/III | I/IV | III/II | III/IV | III/IV |
|----------|------|-------|------|--------|--------|--------|
| Age (years) | 1.001 | 0.002 | 0.001 | 1.001 | 0.0001 | 0.001 |
| Smoking index | 0.37 | 0.01 | 0.001 | 1.001 | 0.001 | 0.001 |
| COPD duration | 0.26 | 0.66 | 0.001 | 1.0003 | 0.001 |
| Triceps | 0.2 | 0.001 | 0.12 | 0.83 | 0.001 |
| Subscapular | 0.007 | 0.26 | 0.001 | 0.001 | 0.001 |
| Suprailiac | 0.01 | 0.3 | 0.001 | 1.0003 | 0.001 |
| MAC | 0.29 | 0.005 | 0.001 | 0.9 | 0.43 | 1 |
| FVC | 1.001 | 0.001 | 1.001 | 0.001 | 0.001 |
| FEV1 pred | 1.001 | 0.001 | 1.001 | 0.001 | 0.001 |
| FEV1/FVC (L) | 0.06 | 0.001 | 0.001 | 1.0001 | 0.001 |
| 6MWT (M) | 1.001 | 0.005 | 0.001 | 0.001 | 0.001 |
| MMP-9*(ng/ml) | 1.03 | 0.33 | 1.26 | 1.1 |
| TIMP1*(ng/ml) | 0.45 | 0.33 | 0.23 | 0.17 | 1 |
| SGRQ score | 1 | 0.001 | 0.001 | 0.001 | 0.001 |
| BODE index | 1 | 0.001 | 0.001 | 0.001 | 0.001 |

**Table 3: Correlation of serum MMP-9 concentration with various parameters (n=200)**

| Correlation | MMP-9 |
|-------------|-------|
| Group I (n=50) | Group II (n=50) | Group III (n=50) | Group IV (n=50) |
| FVC | -0.2 | 0.3 | -0.24 | 0.09 | -0.07 | 0.6 | 0.2 | 0.2 |
| FEV1 (L) | -0.5 | 0.7 | -0.3 | 0.05 | -0.12 | 0.4 | 0.2 | 0.09 |
| 6MWT (m) | -0.3 | 0.03 | -0.2 | 0.26 | -0.04 | 0.8 | 0.2 | 0.12 |
| Smoking index | -0.06 | 0.7 | 0 | 0 | -0.4 | 0.005 | 0 | 0 |
| BODE index | 0.11 | 0.4 | 0.23 | 0.0 | -0.2 | 0.2 | -0.3 | 0.02 |
| BMI | 0.2 | 0.3 | -0.23 | 0.09 | 0.2 | 0.2 | 0.15 | 0.3 |
| Biceps | 0.2 | 0.2 | -0.11 | 0.44 | 0.2 | 0.13 | 0.15 | 0.3 |
| Triceps | 0.3 | 0.04 | 0.04 | 0.80 | 0.3 | 0.05 | 0.07 | 0.6 |
| Subscapular | 0.2 | 0.2 | -0.2 | 0.29 | 0.9 | 0.2 | 0.13 | 0.4 |
| Suprailiac | 0.2 | 0.2 | -0.08 | 0.54 | 0.24 | 0.08 | 0.09 | 0.3 |
| MAC | 0.2 | 0.2 | -0.2 | 0.12 | 0.08 | 0.6 | 0.11 | 0.4 |
| QOL | -0.01 | 0.9 | 0.3 | 0.06 | 0.02 | 0.9 | -0.07 | 0.6 |
| COPD duration | -0.12 | 0.4 | 0.08 | 0.55 | 0 | 0 | 0 | 0 |

Proteases are responsible for the destruction of lung parenchyma (tissue remodeling and repair) while the antiproteases exert a protective effect by binding to MMP-9 and inhibiting its enzymatic activity. Recent evidence suggests that excess proteolytic activity over the inhibitory capacity of the lung leads to parenchymal destruction and development of emphysema.[18]

Studies of human samples have shown an increase in many proteases, including MMP in smoking-related emphysema. Several MMPs including MMP-1, MMP-2, MMP-8, MMP-9, and MMP-12 are elevated both in experimental emphysema and human COPD.[7,13,14,18,19] Of these, MMP-8, MMP-9, and MMP-12 have been especially found to be associated with COPD.[20-23]

In the present study, no significant differences were found in serum MMP-9 levels of patients with COPD (smokers as well as nonsmokers) compared to those without COPD, although in absolute terms, COPD patients had higher values. This indicates the presence of increased systemic inflammatory response in patients with COPD compared to those without. Previous studies have shown that MMP-9 levels were elevated in sputum and sera of patients with COPD and also help to discriminate between symptomatic smokers and COPD patients.[12-14,24-28] In contrast, however, a recent study found lower levels of MMP-9 and TIMP-1 in the plasma of patients with emphysema compared to smokers without COPD and nonsmoking controls although corresponding values in BAL fluid were higher.[29] This might indicate a possible discordance between the local and systemic inflammatory milieu in COPD.
Table 4: Correlation of TIMP-1 concentration in serum with various parameters (n=200)

| Correlation          | Group I (n=50) | Group II (n=50) | Group III (n=50) | Group IV (n=50) |
|----------------------|----------------|----------------|-----------------|----------------|
|                      | r   | P value | r   | P value | r   | P value | r   | P value |
| FVC                  | 0.11| 0.43   | -0.13| 0.4     | -0.07| 0.63   | -0.3| 0.063  |
| FEV1(L)              | 0.09| 0.52   | -0.10| 0.5     | -0.09| 0.52   | -0.32| 0.023  |
| 6MWT (m)             | -0.29| 0.03   | -0.33| 0.02    | -0.45| 0.0009 | -0.5| 0.0002 |
| Smoking index        | 0.21| 0.12   | 0    | 0       | 0.13| 0.35   | 0   | 0      |
| BOGE index           | 0.15| 0.28   | 0.12| 0.4     | 0.11| 0.41   | 0.5| 0.0007 |
| BMI                  | 0.13| 0.34   | -0.10| 0.5     | 0.07| 0.58   | -0.15| 0.3    |
| Biceps               | 0.07| 0.58   | -0.14| 0.31    | 0.15| 0.28   | -0.007| 0.95  |
| Triceps              | 0.29| 0.03   | 0.19| 0.18    | 0.25| 0.08   | 0.2| 0.2    |
| Subscapular          | 0.14| 0.33   | 0.03| 0.80    | 0.16| 0.24   | 0.2| 0.2    |
| Suprailiac           | 0.20| 0.14   | -0.07| 0.63    | 0.34| 0.02   | 0.02| 0.9    |
| MAC                  | 0.17| 0.22   | 0.08| 0.56    | 0.04| 0.77   | -0.03| 0.83   |
| QOL                  | -0.04| 0.77  | 0.06| 0.66    | 0.22| 0.12   | -0.11| 0.42   |
| COPD duration        | 0.03| 0.84   | 0.23| 0.11    | 0   | 0      | 0   | 0      |

Data are shown in Spearman’s rank correlation coefficient $r$ ($r$ values), $P$ value $<0.05$ taken as significant and $<0.001$ highly significant.

These findings imply that not only is exercise capacity poor in COPD patients but also is probable that systemic inflammation has a role to play in this impairment of activity through the increased production of inflammatory cytokines during skeletal muscle activity.

A significant negative correlation was observed between MMP-9 and the degree of airway obstruction as measured by FEV1, thereby favoring a possible pathogenic direct role of MMP-9 in causing airway obstruction, even though similar correlation was not seen with the composite BODE index. Increased MMP-9 and TIMP-1 and inverse relation to airway obstruction favor the concept that MMP-9/TIMP-1 imbalance causes greater degree of airway obstruction.

Similarly, TIMP-1 levels showed a trend of elevation, although insignificantly, in COPD patients (Groups I and II) compared with Groups III and IV. The highest TIMP-1 values were observed in Group II (nonsmoking COPD patients) implying perhaps that the dysfunctional matrix remodeling is more active in COPD irrespective of the presence or absence of tobacco exposure compared to non-COPD smokers and nonsmokers. The relationship between MMP-9 and TIMP-1 has not always been constant. In fact, MMP-9 levels have actually been observed to increase following 3–6 months of smoking cessation although TIMP-1 remains constant. This may explain the role of MMP-9 in continued pulmonary damage predisposing to COPD.

It is noteworthy that the differences in MMP-9 and TIMP-1 between Groups I and II (tobacco vs. nontobacco exposed COPD) were marginal. This suggests that the degree of inflammation as indicated by these markers is not directly affected by the possible causative agent for COPD. In addition, neither MMP-9 nor TIMP-1 demonstrated significant correlation with disease severity (assessed by the BODE index) in COPD due to either tobacco or nontobacco etiology. Similar findings were observed in a previous study on 101 patients with emphysema, wherein no association was observed between MMP-9 or TIMP-1 with disease severity, progression, or FEV1 decline over a 6-month period. These findings imply that protease-antiprotease balance is not proportional to disease severity and unlikely to be a reliable prognostic marker.

Among the parameters of physical activity, the 6-MWD was lower in COPD patients compared to non-COPD smokers or healthy subjects and it negatively correlated with COPD duration. This may explain the relation between QOL and systemic inflammation has a role to play in this impairment of activity through the increased production of inflammatory cytokines during skeletal muscle activity.

We did not note any gender difference in levels of MMP-9 and TIMP-1, a finding similar to that reported recently in a cohort of COPD patients as well as non-COPD smokers. However, patients with COPD (Groups I and II) were significantly more cachectic and malnourished compared to non-COPD patients as evident from the anthropometric parameters and BMI. Malnutrition is a well-known observation in COPD and presumes to be a relatively common systemic manifestation with a prevalence ranging up to 47.2%. Similarly, the markedly worse QOL in COPD compared to non-COPD is well known although the relation between QOL and systemic inflammation has been less studied. No conclusive association between QOL and MMP-9 or TIMP-1 could be observed in our patients as well.

**CONCLUSIONS**

COPD is associated with worse muscle mass and QOL compared to non-COPD counterparts. The protease-antiprotease balance in COPD is similar irrespective of the presence or absence of tobacco exposure but is related to poor exercise capacity.

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**Conflicts of interest**

There are no conflicts of interest.

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