Expression of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, Tissue Inhibitor of Metalloproteinase-1, and Changes in Alveolar Septa in Patients with Chronic Obstructive Pulmonary Disease

Authors' Contribution:
Study Design **A**
Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
Manuscript Preparation **E**
Literature Search **F**
Funds Collection **G**

ABCDE **Yongxiang Zhang**
AEFG **Yuechuan Li**
BD **Zhen Ye**
CF **Hui Ma**

Department of Respiratory and Critical Care Medicine, Tianjin Chest Hospital, Tianjin, P.R, China

Corresponding Author: Yuechuan Li, e-mail: liyuechuandoctor1@163.com
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Background: This study investigated the relationship between the pathological alteration of alveolar septa and (1) pulmonary function and (2) matrix metalloproteinase (MMP)-2, MMP-9, and tissue inhibitor matrix metalloproteinase 1 (TIMP-1) expression in chronic obstructive pulmonary disease (COPD).

Material/Methods: Sixty patients with pulmonary disease were divided into control (n=20) and COPD (n=40) groups. Postoperative lung tissue specimens were examined. Hematoxylin and eosin and elastin van Gieson staining detected pathological alterations of pulmonary alveolar septa. Septa thickness was measured. MMP-2, MMP-9, and TIMP-1 expression levels were detected by immunohistochemical staining. Correlations were determined by Pearson analysis.

Results: Forced expiratory volume in 1 s (FEV_1), forced vital capacity, FEV_1 percent predicted (FEV_1%pre), and diffusion capacity of carbon monoxide percent predicted (DLCO%pre) in COPD patients were significantly lower than in those of the control group ($P<0.05$). MMP-2, MMP-9, and TIMP-1 expression levels were significantly higher in the COPD group than in control, especially the severe group ($P<0.05$). Septa thickness was negatively correlated with FEV_1%pre ($r=-0.335$; $P<0.05$) and positively correlated with MMP-2 and TIMP-1 expression ($P<0.05$). Proportion of collagenous fiber was negatively correlated with FEV_1%pre and DLCO%pre ($P<0.01$), and positively correlated with MMP-2, MMP-9, and TIMP-1 expression ($P<0.01$). Proportion of elastic fibers was negatively correlated with collagenous fiber.

Conclusions: The pathological alteration of alveolar septa was correlated with pulmonary function and expression levels of MMP-2, MMP-9, and TIMP-1, which can play vital roles in COPD progression.

MeSH Keywords: **Matrix Metalloproteinase 9 • Pathological Conditions, Signs and Symptoms • Respiratory Function Tests**

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Background

Chronic obstructive pulmonary disease (COPD) is a common chronic illness marked by persistent respiratory symptoms and restricted airflow. It is caused by abnormalities of the airway or alveolar septa due to significant exposure to harmful particles or gases [1,2]. COPD is a complex disease that includes many clinical subtypes, such as the non-exacerbator phenotype, frequent exacerbator phenotype with emphysema, exacerbator phenotype with chronic bronchitis, and asthma-COPD overlap syndrome [3,4]. COPD severely affects human health and quality of life and ranks as the fourth leading cause of death worldwide [5]. Furthermore, COPD carries a significant financial burden worldwide [6]. In China, the prevalence of COPD among 40-year-olds is about 10% [7]. Therefore, it is important to study the pathogenesis and pathological changes of COPD to effectively prevent and treat the disease.

The expiratory movement during calm breathing is mainly produced by the relaxation of the diaphragm and external intercostal muscles and the elastic retraction of the lungs pulling the thoracic cage [8]. The increase of pulmonary volume in COPD patients is caused by the increase of expiratory resistance caused by airway lesions and an increase in residual air volume, while the decreased elastic retraction force of the lungs also has a very important role in the increase of pulmonary volume in patients with COPD [9]. The forced expiratory volume in 1 s percent predicted (FEV_1%pre) and the carbon monoxide diffusing capacity percent predicted (DLCO%pre) are important indices of lung function, and the values of both FEV_1%pre and DLCO%pre are decreased in patients with lung disease [10–12]. Previous studies on the pathological changes of COPD have primarily focused on the pathological changes of the airway, especially the small airway [13,14]. Studies have shown that vasculitis, vascular endothelium structural and functional changes, vascular remodeling, pulmonary lymphangiectasis, and an increase in the number of lobular lymphatic vessels are seen in the pulmonary interstitium of patients with COPD [15–17]. Bronchial angiogenesis and pulmonary vascular remodeling are increased, eventually leading to changes in bronchial and pulmonary circulation [18]. However, the mechanism of the pathological changes of pulmonary interstitial alveolar septa in patients with COPD has not been well characterized. Studies have found that the majority of patients with later-stage COPD have concomitant pulmonary interstitial fibrosis, aggravating the occurrence of persistent airway obstruction and ventilation disorders [19,20]. The alveolar walls are composed of a monolayer of squamous epithelium, including squamous epithelium (type I cells), secretory epithelium (type II cells), and septal cells. The alveolar septum, a structure between adjacent alveolar walls, consists of connective tissue and abundant capillaries. Because the alveolar septa are a significant part of the pulmonary interstitium,

it is important to study their pathological changes in COPD to identify the pathological basis of the disease.

Matrix metalloproteinases (MMPs) are a set of enzymes which can degrade components of the extracellular matrix (ECM) [21]. MMP-2 and MMP-9 act on extracellular basement membrane turnover by preferentially degrading type IV collagen, a principal constituent of the basement membrane, thereby affecting the structural integrity of the pulmonary tissue [22,23]. Furthermore, a study showed that serum MMP-2, MMP-9, and MMP-12 levels were significantly increased in patients with COPD and positively correlated with disease severity [24]. Tissue inhibitor matrix metalloproteinase (TIMP) is a tissue depressor of MMPs and a particular depressor of MMP activity. Because MMPs play an important role in the pathogenesis and development of COPD, we further investigated the relationship between lung function and MMP-2, MMP-9, and TIMP-1 levels.

The aim of this study was to explore the relationship between pathological changes of alveolar septa in the pulmonary interstitium with pulmonary function and local expression levels of MMP-2, MMP-9, and TIMP-1 in patients with COPD.

Material and Methods

Patients

The study included 60 patients with pulmonary disease who were admitted to our hospital from May 2014 to December 2015 and underwent pulmonary lobectomy. All patients were carefully evaluated before surgery. Inclusion criteria were as follows: (1) pulmonary lesion ≤3 cm; (2) preoperative pulmonary function and chest high-resolution computed tomography (HRCT) examinations; (3) age between 50 and 70 years; and (4) Han ethnicity. Exclusion criteria were as follows: (1) history of bronchial asthma, pulmonary fibrosis, bronchiectasis, or tuberculosis; (2) history of connective tissue disease; (3) HRCT of the chest showed pulmonary interstitial lesions; and (4) incomplete clinical data.

The basic patient characteristics, including sex, age, and smoking history and smoking index, were collected. Smoking index=number of cigarettes smoked per day × years of smoking. Simultaneously, the preoperative pulmonary function measures (pulmonary function indicators after the dilation test) were collected, including FEV_1/forced vital capacity (FVC), FEV_1%pre, DLCO%pre, and pathological biopsy results. Ethics approval for the study was obtained from the Medical Ethics Committee of the Tianjin Chest Hospital. Written informed consent was collected from all participants in the study.
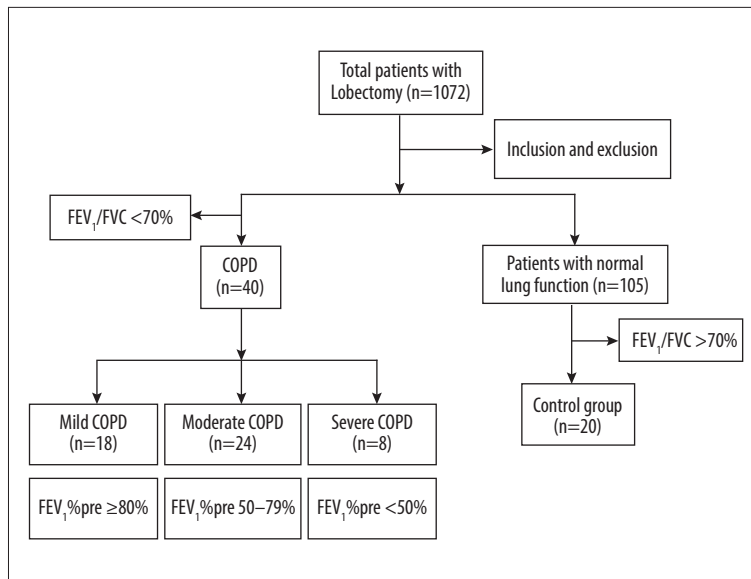
Figure 1. Flow diagram of patient enrollment.

The patients were grouped according to preoperative pulmonary function as follows: the control group was defined as FEV_1/FVC >70%; the COPD group was defined as FEV_1/FVC <70%. According to the value of FEV_1%pre, the COPD group was further divided into 3 groups: the mild COPD group was defined as FEV_1%pre ≥80%; the moderate COPD group was defined as FEV_1%pre 50–79%; and the severe COPD group was defined as FEV_1%pre <50%. The flow diagram of patient enrollment is shown in Figure 1.

Collection and preparation of pathological specimens

The lung tissue samples analyzed in this study were postoperative lung tissue specimens of patients with lung tumors. Pathological specimens of patients after pulmonary lobectomy were collected by automatic microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany). Specifically, for patients who met the inclusion and exclusion criteria, pathological specimens (10×5×5 mm) were excised from near the visceral pleura at a distance of >2 cm from the lesion. Specimens were dehydrated and embedded in paraffin wax and numbered. The thickness of the lung alveolar septa on light microscopy examination was measured by a stage micrometer measurement and image analysis software. Briefly, under a high-power microscope (10×40), 5 alveolar images were randomly selected from each specimen and the thicknesses of the alveolar septa was measured at 45° intervals (a total of 8 parts) with the center of each alveolar as the center. When a local measurement of one part was not possible, it was discarded and all other parts were used. The mean value of the alveolar septal thickness was then measured. The thickness of the specimens was 4 um, and 10 specimens were prepared for each sample. All specimens were prepared for hematoxylin and eosin (H&E) staining, elastin van Gieson (EVG) staining, and immunohistochemistry.

H&E staining

An H&E staining kit (G1120, Solarbio, Beijing, China) was used to stain the tissue sections. The sections were deparaffinized in xylene twice (2–5 min each time), and rehydrated with successive washes in 100% ethanol for 1 min, 95% ethanol for 1 min, 95% ethanol for 2 min, 75% ethanol for 1 min, tap water for 2 min, and distilled water for 2 min. The tissue sections were then stained with hematoxylin for 5 min, rinsed with distilled water for 1 min, differentiated with 75% hydrochloric acid ethanol for 30 s, rinsed with 50°C tap water for 5 min, rinsed with distilled water for 5 min, then stained with eosin for 1-2 min, and rinsed again with distilled water. Furthermore, sections were dehydration with graded alcohol: 95% ethanol (2×1 min), 100% ethanol (2×1 min), and clearing in xylene (2×1 min). The mounted slides were then examined and photographed using an Olympus BX50 optical microscope (Olympus, Tokyo, Japan) with an ISCapture microscope imaging system (Tucsen Photonics). The staining intensity was analyzed by Image-Pro Plus 5.0 software and expressed as an integrated optical density value. Ten fields were randomly selected at 100× magnification for each section.

EVG staining

The tissue sections were deparaffinized in xylene twice (2-5min each time), rehydrated, and then successively washed in 100% ethanol for 1 min, 95% ethanol for 1 min, 95% ethanol for 2 min, 75% ethanol for 1 min, tap water for 2 min, and distilled water for 2 min. The sections were then oxidized with potassium permanganate for 5 min, washed in distilled water, whitened with oxalic acid for 5 min, and washed in distilled water. Tissue sections were then stained with elastin for 8–24 h, and then differentiated with 95% ethanol. After washing thoroughly

Table 1. Analysis of general information of patients.

Group	Control (n=20)	COPD (n=40)	P value
Male (n, %)	13 (65.00%)	29 (72.50%)	0.36
Age (years)	58.05±9.33	60.50±4.30	1.12
Smoking history (n, %)	7 (35.0%)	16 (40.00%)	0.14
Smoking index	165.00±153.13	167.50±152.56	0.06
Emphysema (n, %)	0 (0.00%)	25 (62.5%)	NA
Pulmonary cancer (n, %)	18 (90.00%)	37 (92.50%)	
Pulmonary hamartoma (n, %)	1 (5.00%)	2 (5.00%)	0.47
Teratoma (n, %)	1 (5.00%)	0 (0.00%)	
Pulmonary lipoma (n, %)	0 (0.00%)	1 (2.50%)	

COPD – chronic obstructive pulmonary disease; DLCO – carbon monoxide diffusing capacity.

with tap and distilled water, van Gieson dye (Solarbio, Beijing, China) was used to stain for 1 min, and 95% ethanol was rapidly differentiated for 10 s. The sections were dehydrated with 95% ethanol (2×1 min), 100% ethanol (2×1 min), and cleared in xylene (2×1 min). The mounted slides were then examined and photographed using the Olympus BX50 microscope with the ISCapture microscope imaging system. Ten fields were randomly selected at 100× magnification for each section.

Immunohistochemical staining

The sections were deparaffinized and rehydrated, and washed with 0.01 M phosphate-buffered saline (PBS) (Servicebio, China) (3×5 min). The sections were then incubated with 0.3% methanol and hydrogen peroxide, incubated for 20 min at room temperature, and then washed with distilled water (3×5 min). The sections were incubated with 10% goat serum for 30 min at 37°C, the excess serum was absorbed by filter paper, and the primary monoclonal mouse antibodies to MMP-2 (Calbiochem-Novabiochem, Schwalbach, Germany; clone 42-5D11; dilution 1: 25), MMP-9 (Calbiochem-Novabiochem; clone 56-2A4; dilution 1: 50), TIMP-1(Calbiochem-Novabiochem; clone 10-2D1; dilution 1: 20) were added, then the sections were washed with 0.01 M PBS (3×5 min). The sections were then exposed to a secondary antibody, biotinylated goat anti-mouse antibody, and then to peroxidase conjugated streptavidin. Next, they were incubated with MMP-2, MMP-9, and TIMP-1 working solution for 30 min at 37°C, then sections were washed with 0.01 M PBS (3×5 min). The sections were next incubated with horseradish peroxidase to mark the working fluid for 30 min at 37°C, then the sections were washed with 0.01 M PBS (3×5 min) followed by diaminobenzidine (DAB) and hematoxylin staining. Next, the slides were examined and photographed using the Olympus BX50 optical microscope with the ISCapture microscope imaging system. The DAB staining was analyzed by Image-Pro Plus 5.0 software. Ten fields were randomly selected at 100× magnification for each section.

Statistics Analysis

SPSS 19.0 (SPSS, Inc, Chicago, IL, USA) statistical software was used for all data analyses, and measurement data were expressed as mean±standard deviation. The independent t test was used for the comparison between the 2 groups, and one-way ANOVA was used for the multi-group comparison followed by the least significant difference test. The staining was analyzed by Image-Pro Plus 5.0 software. Correlations between pulmonary function and pathological changes of the alveolar septa were analyzed by Pearson correlation analysis. P value <0.05 was considered statistically significant.

Results

Analysis of clinical characteristics

A total of 60 patients who were admitted from May 2014 to December 2015, had pulmonary disease, and underwent pulmonary lobectomy were recruited into the study (Table 1). The patients were divided into 2 groups according to their levels of preoperative pulmonary function: the control group (n=20) and the COPD group (n=40). The control group had an average patient age of 58.05±9.33 years and included 18 patients with pulmonary cancer, 1 patient with pulmonary hamartoma, and 1 patient with teratoma. A total of 13/20 patients were men (65%). The COPD group had an average patient age of 60.50±4.30 years and included 37 patients with pulmonary cancer, 2 patients with pulmonary hamartoma, 1 patient with pulmonary lipoma, and 25 patients with emphysema. A total of 29/40 patients were men (72.5%). Patient sex, age, smoking history and smoking index and patient types in 2 groups showed no significant differences (P>0.05) between groups. Patients with pulmonary malignant tumor staging were type I. In addition, the exacerbation rate in 1 year and the Global

Table 2. The exacerbation rate in 1 year and GOLD groups in the patients with COPD.

	Mild (n=8)	Moderate (n=24)	Severe (n=8)
Exacerbation rate in 1 year (n, %)			
None	7 (17.5)	15 (37.5)	3 (7.5)
Once	1 (2.5)	6 (15)	2 (5)
Twice	0 (0)	3 (7.5)	2 (5)
Thrice	0 (0)	0 (0)	1 (2.5)
GOLD groups (n, %)			
A	7 (17.5)	12 (30)	0
B	1 (2.5)	9 (22.5)	5
C	0 (0)	2 (5)	1 (2.5)
D	0 (0)	1 (2.5)	2 (5)

GOLD – Global Initiative for Obstructive Lung Disease.

Table 3. Comparison of pulmonary function between COPD group and control group.

Groups	Control (n=20)	COPD			
		Total (n=40)	Mild (n=8)	Moderate (n=24)	Severe (n=8)
FEV_1 (L)	3.56±0.74	2.11±0.57**	2.24±0.12**	2.10±0.16**	2.03±0.17**
FVC (L)	4.09±0.84	3.60±0.17**	3.66±0.54	3.60±0.56*	3.53±0.68**
FEV_1/FVC (%)	87.26±2.60	60.16±7.93**	61.95±6.58**	59.49±8.83**	60.38±671**
FEV_1%pre	90.30±5.07	63.71±2.20**	82.30±3.07*	63.33±7.53**,##	45.25±3.71**,##,&&
DLCO%pre	84.96±13.03	75.83±15.30*	81.68±10.03	78.65±14.98	61.51±12.83**,##,&&

FEV_1 – forced expiratory volume in 1 second; FVC – forced vital capacity; DLCO – carbon monoxide diffusing capacity; *vs.* Control group, * $P<0.05$, ** $P<0.01$; *vs.* Mild group, ## $P<0.01$; *vs.* Severe group, && $P<0.01$.

Initiative for Obstructive Lung Disease (GOLD) groups of the patients with COPD are shown in Table 2.

Comparison of pulmonary function

Differences in pulmonary function in the control and COPD groups were analyzed (Table 3). According to the FEV_1%pre value, the COPD group was further divided into a mild COPD group (n=8), a moderate COPD group (n= 24), and a severe COPD group (n=8). FEV_1, FVC, FEV_1/FVC, FEV_1%pre, and DLCO%pre of patients in the COPD group were was all significantly lower compared to those in patients without COPD ($t=-11.83$, $P<0.01$; $t=-2.67$, $P<0.01$; $t=-14.84$, $P<0.01$; $t=-2.29$, $P=0.03$, respectively), and were decreased with the decrease in COPD severity. In particular, the severe group showed the lowest FEV_1%pre (45.25±3.71) and DLCO%pre (61.51±12.83), which were significantly lower in the severe group than in the mild and moderate COPD groups and the control group ($P<0.01$).

Pathological analysis of alveolar septa

The results of H&E and EVG staining showed disordered internal fibers and increased alveolar rupture in the COPD group in comparison with those of the control group (Figure 2). The alveolar septa thickness and the proportion of collagen fibers were significantly higher in the COPD group ($t=2.29$, $P=0.03$; $t=2.54$, $P=0.01$, respectively), while the proportion of elastic fibers and elasticity/collagen were significantly lower ($t=-2.49$, $P=0.02$; $t=-3.70$, $P<0.01$, respectively) (Table 4). Notably, in the severe group, the thickness and the proportion of collagen fibers were markedly higher ($P<0.01$), while the proportion of elastic fibers and elasticity/collagen were significantly lower compared to those of the control, mild, and moderate groups ($P<0.05$). Taken together, these results indicated that the pathological change in the pulmonary interstitium disease state developed progressively with the development of COPD disease, which was associated with the severe rupture of alveolar septa.
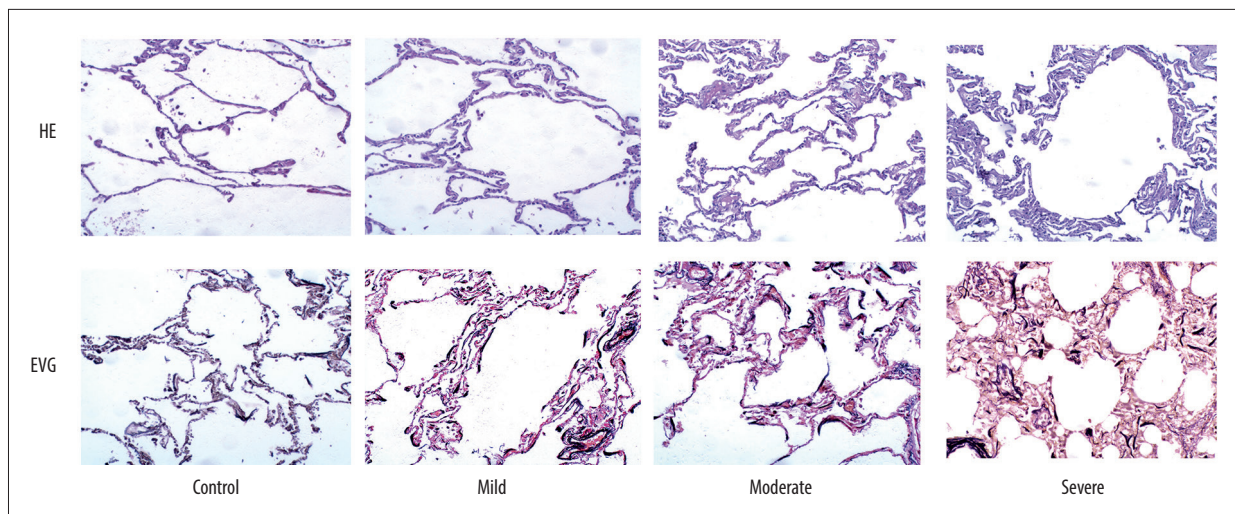
Figure 2. Comparison of alveolar septa thickness and fiber morphology (H&E, ×100 magnification; EVG, ×100 magnification).

Table 4. Comparison of alveolar septum thickness and fiber ratio between COPD group and control group.

Groups	Control (n=20)	COPD			
		Total (n=40)	Mild (n=8)	Moderate (n=24)	Severe (n=8)
Thickness(um)	5.63±1.87	6.90±2.11*	6.19±1.65	6.44±1.75	9.00±2.39**,##,&&
Collagenous fiber (%)	39.26±10.55	47.60±12.62*	40.88±13.24	45.88±9.64	59.50±13.52**,##,&&
Elastic fibers (%)	45.31±8.08	37.43±12.96*	40.38±11.70	39.63±13.26	27.88±9.51**,#,&
Elasticity/collagen	108.54±37.85	82.87±38.85*	107.55±41.10	88.86±35.13	48.38±17.97**,##,&&

COPD – chronic obstructive pulmonary disease; DLCO – carbon monoxide diffusing capacity; *vs.* Control group, * $P<0.05$, ** $P<0.01$; *vs.* Mild group, # $P<0.05$, ## $P<0.01$; *vs.* Severe group, & $P<0.05$, && $P<0.01$.

**Table 5.** Correlation between alveolar septum thickness and fiber composition ratio and lung function.

r (P)	Linear correlation coefficient		
	Thickness (um)	Collagenous fiber (%)	Elastic fibers (%)
FEV_1	−0.245 (0.06)	−0.360 (0.005)	0.231 (0.076)
FVC	−0.206 (0.115)	−0.224 (0.085)	−0.051 (0.699)
FEV_1/FVC	−0.170 (0.193)	−0.267 (0.04)	0.373 (0.003)
FEVv%pre	−0.335 (0.035)	−0.472 (0.002)	0.316 (0.047)
DLCO%pre	−0.078 (0.554)	−0.329 (0.010)	0.316 (0.014)

FEV_1 – forced expiratory volume in 1 second; FVC – forced vital capacity; DLCO – carbon monoxide diffusing capacity.

Relationship between pathological changes of alveolar septa and pulmonary function

As shown in Table 5, the relationship between the pathological changes of the alveolar septa and lung function was analyzed. Pearson correlation analysis revealed a significant negative relationship between collagenous fiber and FEV_1 ($r=−0.360$; $P=0.005$) and FEV_1/FVC ($r=−0.267$; $P = 0.046$). A significant positive relationship between FEV_1/FVC and elastic fibers proportion ($r=0.373$; $P=0.003$) was observed. Significant negative relationships between FEV_1%pre and alveolar septa thickness and collagen fibers ($r=−0.335$; $P=0.035$; $r=−0.472$, $P=0.002$, respectively) and a significant positive relationship between FEV_1%pre and elastic fiber proportion ($r=0.316$, $P=0.047$) were observed. The change in fibers could have affected the ventilatory capacity of patients with COPD. Meanwhile, DLCO%pre showed a significant negative relationship between lung function and collagen fibers ($r=−0.329$, $P=0.010$) and a significant positive
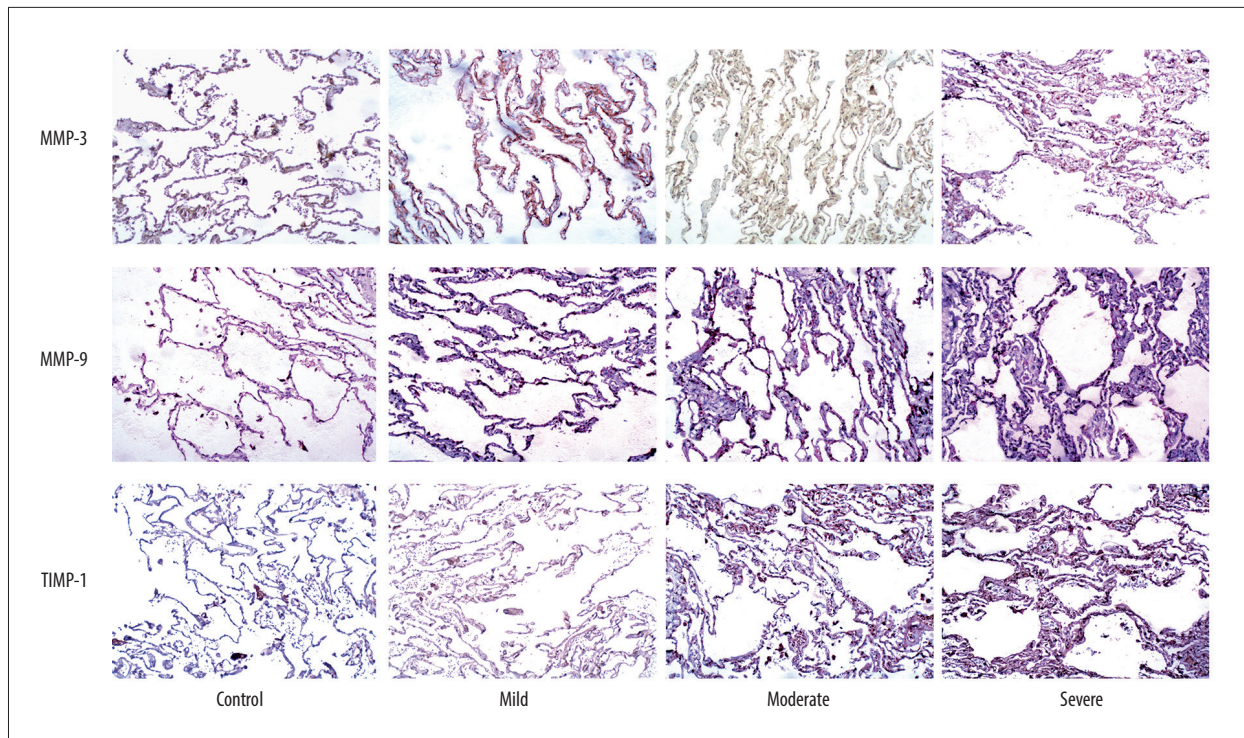
Figure 3. Immunohistochemical analysis of the expression of matrix metalloproteinase (MMP)-2, MMP-9, and tissue inhibitor of metalloproteinase (TIMP)-1 in alveolar septa (×100 magnification).

Table 6. Comparison of expression of MMP-2, MMP-9 and TIMP-1 in alveolar septa between COPD group and control group.

Groups	Control (n=20)	COPD			
		Total (n=40)	Mild (n=8)	Moderate (n=24)	Severe (n=8)
MMP-2	0.11±0.02	0.15±0.04**	0.13±0.02**	0.16±0.02**,##	0.22±0.06**,#
MMP-9	0.11±0.02	0.15±0.03**	0.122±0.03	0.16±0.02**,##	0.19±0.02**,##
TIMP-1	0.11±0.03	0.14±0.06**	0.10±0.03	0.13±0.03*	0.24±0.05**,##,&&
MMP-2/TIMP-1	1.05±0.24	1.10±0.26	1.15±0.32	1.03±0.16	0.91±0.37##
MMP-9/TIMP-1	1.08±0.41	1.17±0.37	1.08±0.41	1.31±0.31*	0.85±0.33*,#,&

COPD – chronic obstructive pulmonary disease; DLCO – carbon monoxide diffusing capacity; MMP – matrix metalloproteinase, TIMP-1 – tissue inhibitor of metalloproteinase-1; $vs.$ Control group, * $P<0.05$, ** $P<0.01$; $vs.$ Mild group, # $P<0.05$, ## $P<0.01$; $vs.$ Severe group, & $P<0.05$, && $P<0.01$.

relationship with elastic fiber proportion (r=0.316, P=0.014). These results revealed that pathological changes of pulmonary interstitial alveolar septa were correlated with lung function changes in patients with COPD. This pathological change could have a negative effect on pulmonary function.

The expression level of MMP-2, MMP-9, and TIMP-1 in patients with COPD

The expression levels of MMP-2, MMP-9, and TIMP-1 in alveolar septa collagen fibers and elastic fibers in the COPD group were significantly higher than those in the control group (Figure 3,

Table 6, t=7.97, $P<0.01$; t=6.33; $P<0.01$; t=2.74, $P<0.01$, respectively). The expression levels of MMP-2, MMP-9, and TIMP-1 in alveolar septa were significantly increased with the progression of COPD, and were the highest in the patients with severe COPD ($P<0.05$). However, the values of MMP-9/TIMP-1 and MMP-2/TIMP-1 were significantly lower in the severe COPD group ($P<0.05$). This indicated that the change of alveolar septa could lead to the expression of MMP-2, MMP-9, and TIMP-1 in alveolar septa, and MMP-2, MMP-9, and TIMP-1 played vital roles in the pulmonary interstitial metabolism of the COPD pathogenesis.

Table 7. Correlation between alveolar septum thickness and fiber composition ratio and the expression of MMP-2, MMP-9, and TIMP-1.

r (P)	Linear correlation coefficient					
	Thickness (um)		Collagenous fiber (%)		Elastic fibers (%)	
MMP-2	0.289	(0.025)	0.353	(0.006)	−0.271	(0.037)
MMP-9	0.130	(0.324)	0.386	(0.002)	−0.268	(0.038)
TIMP-1	0.417	(0.001)	0.563	(0.001)	−0.295	(0.022)
MMP-2/TIMP-1	0.163	(0.215)	−0.120	(0.36)	0.045	(0.733)
MMP-9/TIMP-1	−0.302	(0.019)	−0.249	(0.055)	0.098	(0.455)

COPD – chronic obstructive pulmonary disease; DLCO – carbon monoxide diffusing capacity; MMP – matrix metalloproteinase; TIMP-1 – tissue inhibitor of metalloproteinase-1.

Relationship between pathological changes of alveolar septa and expression of MMP-2, MMP-9, and TIMP-1

As shown in Table 7, the results of the correlation analysis showed that the thickness of alveolar septa was positively correlated with the expression levels of MMP-2 and TIMP-1 (r=0.289, P=0.025; r=0.417, P=0.001, respectively) and negatively correlated with the ratio of MMP-9/TIMP-1 (r=−0.302, P=0.019). The proportion of collagenous fiber was positively correlated with the expression levels of MMP-2, MMP-9, and TIMP-1 (r=0.353, P=0.006; r=0.386, P=0.002; r=0.563, P=0.001, respectively). Moreover, negative correlations were observed between the proportion of elastic fibers and the expression of MMP-2, MMP-9, and TIMP-1 (r=−0.271, P=0.037; r=−0.268, P=0.038; r=−0.295, P=0.022, respectively). The pathological changes of the pulmonary interstitial alveolar septa were related to MMP-2, MMP-9, and TIMP-1 in patients with COPD.

Discussion

COPD is a class of airway inflammatory diseases, and smoking is its most commonly recognized risk factor [6]. COPD is a highly endemic and heterogeneous disease with high morbidity and mortality, placing a heavy burden on health care providers worldwide. Treatment options that confer a mortality benefit to the patient are rare. Research on the pathogenesis and pathological changes of COPD is crucial to explore the effective prevention and treatment of the disease. However, to date, the pathogenesis of COPD is still not clear. In the present study, we investigated the relationship between the pathological alterations of the alveolar septa and lung function, and the levels of MMP-2, MMP-9, and TIMP-1 in patients with COPD.

In the present study, sex, age, and smoking index did not differ significantly in the 2 groups. The DLCO%pre and FEV$_1$%pre values of the COPD group were significantly lower than that of the control group, and the severe COPD group showed markedly lower levels than in the mild and moderate COPD groups.

Our results indicated that the pulmonary diffusion function in patients with COPD was lower, which is in agreement with a previous study [25]. Studies have demonstrated that patients present with a decrease in DLCO when alveolar septa and alveolar capillaries are damaged [26,27]. Furthermore, the decrease of DLCO in patients with COPD in the present study revealed that the patients' alveolar septa may have changed. Also, alveolar septa rupture was increased in the COPD group in the present study. The reason might be that, with the restriction of airflow, the alveoli expand significantly, then the alveoli and alveolar septa rupture [28,29].

The fibrous components of the pulmonary interstitium include primarily elastic fibers and collagen fibers, which together form the elastic resistance of the lungs [30]. When the lungs expand, these fibers stretch and tend to retract. Collagen fibers are tough and can resist a pulling force. Elastic fibers give the lungs good elasticity, and the destruction of pulmonary elastic fibers leads to a decrease in the elastic retraction of the lungs. In the present study, we found that the alveolar septa were significantly thicker in patients with COPD, especially those with severe COPD. At the same time, pulmonary alveolar rupture occurred in patients with COPD. The reason may be that in patients with COPD, especially in those who have emphysema, the bronchial lumen dilates when inhaling, the air enters the alveoli, and the pulmonary cavity shrinks when exhaling. The air then remains, and the pressure in the alveoli increases continuously, leading to rupture of the alveolar walls. Furthermore, the alveolar septum thickens, elastic fibers decrease, and the alveolar walls are prone to rupture.

We further investigated the relationship between lung function and pathological changes. With the increase of alveolar septa thickness, the DLCO%pre and FEV$_1$%pre levels were decreased, and FEV$_1$%pre was decreased significantly. The reason can be that the alveolar septum is the nodular tissue between adjacent alveoli, which contains a capillary network, and the thickening of the alveolar septum will inevitably lead to an increase in the gas diffusion distance and reduce the

pulmonary function of patients with COPD [31,32]. Furthermore, collagen fibers were increased in patients with COPD, and collagen fibers in the severe COPD group were significantly increased compared with that of the mild and moderate groups. Similarly, the DLCO%pre and FEV$_1$%pre levels decreased with the increase of collagen fibers. A reason for this may be that the proportion of collagen fibers increased and the proportion of elastic fibers decreased, resulting in the decrease of pulmonary compliance and pulmonary elastic retraction [33,34]. The alteration of collagen fibers and elastic fibers raises the possibility of a remodeling process occurring in the connective matrix in alveolar walls, and connective tissue changes are part of the pathological events in emphysema [35]. Ultimately, airflow limitation in patients with COPD is more obvious, indicating that this pathological change in the alveolar septa may be a pathogenic mechanism in these patients. Similarly, the amount of collagen fibers in alveolar septa was increased in a model of COPD [36].

MMPs have been considered as latent therapeutic targets in a variety of pathological conditions involving abnormal MMP expression and activity [37]. The deposition of ECM has a crucial role in the development process of pulmonary fibrosis. However, ECM is primarily resolved by MMPs, and the conversion efficiency of MMPs is adjusted mainly by TIMPs [38]. It has been shown that the imbalance between MMPs and TIMPs can be an important mechanism in pulmonary fibrosis [39]. Meanwhile, the levels of MMPs and TIMPs were shown to be revised in pulmonary fibrosis [40,41]. Li et al. discovered that the expression and activity of MMPs, especially MMP-2 and MMP-9, were significantly increased in pulmonary tissues after injury [42]. MMP-9 and TIMP-1 expression can serve not only as the indicator to determine the pathological classification, metastasis, and prognosis of carcinoma, but can also help the targeted gene therapy of carcinoma [43,44]. In the present study, expressions levels of MMP-2 and MMP-9 were also significantly higher in patients with COPD. Also, the expression level of TIMP1 was notably increased in patients with COPD, while the values of MMP-2/TIMP-1 and MMP-9/TIMP-1 were increased, in particular the value of MMP-9/TIMP-1. Furthermore, the expression levels of MMP-2, MMP-9, and TIMP-1 in the alveolar septa were significantly higher in the severe COPD group, while the values of MMP-9/TIMP-1 and MMP-2/TIMP-1 were significantly lower in the severe COPD group. Smoking decreases the MMP-9/TIMP-1 ratio [45]. Mohamed et al. found that MMP-9 and TIMP-1 play important roles in the pathophysiology of asthma exacerbation and airway remodeling [46]. Uysal et al. found that MMP-9 concentration and the MMP-9/TIMP-1

ratio are the best predictors of emphysema in patients with COPD [47]. Overall, the results of that experiment are the same as those reported in an earlier study [42]. Yang et al. found the expression levels of MMP-2, MMP-9, and TIMP-1 were significantly upregulated in the pulmonary tissue of rats with pulmonary fibrosis [38]. Similarly, in the present study, we found that collagenous fiber and elastic fibers were correlated with the expression levels of MMP-2, MMP-9, and TIMP-1 in patients with COPD. We also found that pulmonary interstitial alveolar septa thickness was positively correlated with the expression of MMP-2 and TIMP-1 in patients with COPD. The enhanced expression of MMP can lead to increased degradation of EMC in the pulmonary interstitium and the destruction of broncho-lung structure. While inhibiting the activity of MMPS, TIMP promotes the increase of collagen synthesis and fibroblast proliferation, and the alveolar septum also thickens during the formation of pulmonary interstitial fibrosis.

The study has the following limitations: (1) the number of cases was small; (2) there are many pathogenic factors and influencing factors that cause COPD; (3) the use of drugs in patients was not considered; and (4) because of the more obvious lung changes in patients with severe COPD, no such patients were included in the study owing to poor lung function. Further studies are needed to assess the influence of these factors on FEV$_1$ and DLCO.

Conclusions

In the present study, there were significant pathologic changes in the alveolar septa of patients with COPD. Alveolar septa rupture, alveolar septa thickness, and collagen fibers were significantly increased, and elastic fibers and elasticity/collagen were significantly decreased, especially in patients with severe COPD ($P<0.01$), while the expression levels of MMP-2, MMP-9, and TIMP-1 were significantly higher in the COPD group. Lung function of patients with COPD was decreased with the pathological changes of alveolar septa, which were demonstrated to be correlated with pulmonary function and the expression levels of MMP-2, MMP-9, and TIMP-1. The expression levels of MMP-2, MMP-9, and TIMP-1 can play vital roles during the development of COPD.

Conflict of interests

None.

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