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ORIGINAL ARTICLE

Clinical association study on the matrix metalloproteinase expression in the serum of patients with connective tissue disease complicated with interstitial lung disease

Hong Chen[1*], Jun Tang[2*], Juhua Liang[3], Dan Huang[1], Chunfeng Pan[1], Sha Liu[1], Xiuri Du[1], Liju Tao[1]

[1]*Department of Rheumatology and Immunology, Affiliated Hospital of Youjiang Medical University For Nationalities, Baise, China*
[2]*Department of Dermatology, Affiliated Hospital of Youjiang Medical University For Nationalities, Baise, China*
[3]*Department of Clinical Laboratory, Affiliated Hospital of Youjiang Medical University For Nationalities, Baise, China*

ABSTRACT

Objectives: This study was implemented to reveal the expression and the clinical correlation of matrix metalloproteinases (MPSs) with connective tissue disease (CTD) complicated with interstitial lung disease (ILD).

Patients and methods: This clinical study was conducted with 260 patients (151 males, 109 females; mean age: 47.3±12.5 years; range, 29 to 67 years) between October 2019 and October 2020. Among the subjects, 100 were CTD patients (CTD group), 80 were CTD patients with ILD (CTD-ILD group) and 80 were healthy individuals (control group). The MMP-2, -3, -7, and -9 levels in the serum of the three groups were detected by enzyme-linked immunosorbent assay.

Results: Serum levels of MMP-3, -7, and -9 in the CTD-ILD group were higher, while the MMP-2 level was lower than those in the CTD group and the control group. The MMP-7 level in the serum of the CTD-ILD group was positively related to C-reactive protein, erythrocyte sedimentation rate, and rheumatoid factor and negatively correlated with immunoglobulin G and complement 3. The MMP-7 expression in the serum was positively correlated with forced expiratory volume in one second (FEV1%), FEV1/forced expiratory volume (FVC), and FVC in CTD-ILD patients. Pearson statistical analysis revealed that there was a significant positive correlation between the MMP-7 expression and the percentage of B cells in the serum of CTD-ILD patients.

Conclusion: Expressions of MMP-3, -7, and -9 are significantly increased in the serum of patients with CTD and related interstitial lung lesions, and the high expression of MMP-7 indicates dynamic lung lesions, which is possible to be used as a possible biomarker for early diagnosis and assessment of disease progression.

Keywords: Connective tissue disease, enzyme-linked immunosorbent assay, lung interstitial disease, matrix metalloproteinase.

Connective tissue disease (CTD) refers to a type of disease that attacks the musculoskeletal system, including joints, muscles, ligaments, and bursa.[1] This disease proves to be associated with the disorder of immune function of the body, and it is mainly manifested in clinical symptoms, such as pain, fever, morning stiffness, rash, and joint swelling, leading to a high disability rate.[2] Interstitial lung disease (ILD) is usually accompanied by CTD, resulting in a poor prognosis, which would significantly affect the quality of life of patients and even endanger their lives. The pathogenesis

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Correspondence: Liju Tao, MD. Department of Rheumatology and Immunology, Affiliated Hospital of Youjiang Medical University For Nationalities, 533099 Baise, China. E-mail: tlj20220217@163.com

** The first two authors contributed equally to this manuscript.*

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of CTD with ILD may be related to infection, immune disorders, genetics, and endocrine and environmental factors, and its clinical diagnosis mainly depends on lung computed tomography examination; thus, it is inconvenient to monitor the changes in disease activity.[3,4]

Matrix metalloproteinases (MMPs) refer to a superfamily of proteases widely distributed in connective tissue. They have similar structures, and their activity is dependent on zinc ions. Their main biological function is to degrade the extracellular matrix. At present, there are as many as 20 MMPs, which are divided into five groups according to the substrates.[5,6] Rheumatic immune diseases have important characteristics, such as extensive inflammatory cell infiltration, extracellular matrix metabolism disorder, and vascular inflammatory injury, and many studies have reported that MMPs are involved in the occurrence and development of rheumatic immune diseases.[7] In addition, it has been suggested that it is possible to regard MMP-7 as a prognostic indicator in idiopathic pulmonary fibrosis, for this protease is involved in the process of interstitial lung lesions, remodeling of lung epithelial cells, and carcinogenesis.[8,9] Both MMP-2 and -9 have a close association with the inflammatory procession of the body and are involved in the development of airway inflammation and pulmonary fibrosis, which are also able to be deemed as biomarkers for scaling alveolar injury degree.[10,11]

In this study, the contents of MMP-2, -3, -7, and -9 in the serum of CTD patients with ILD were determined, and the results were compared with CTD patients without ILD healthy individuals. The significance of MMPs in CTD complicated with pulmonary interstitial lesions was preliminarily discussed through correlation analysis of these data with the laboratory and clinical indicators of disease activity, providing a basis for clinical diagnosis, disease monitoring, and activity evaluation.

PATIENTS AND METHODS

This clinical study was conducted with 260 patients (151 males, 109 females; mean age: 47.3±12.5 years; range, 29 to 67 years) at the Affiliated Hospital of Youjiang Medical College for Nationalities between October 2019 and October 2020. Among the subjects, 100 were CTD patients (CTD group), 80 were CTD patients with ILD (CTD-ILD group) and 80 were healthy individuals (control group). Inclusion criteria for the CTD group were as follows: *(i)* the patient's symptoms met the diagnostic criteria for rheumatoid arthritis, systemic lupus erythematosus, polymyositis, Sjögren's syndrome, and systemic sclerosis; *(ii)* the patient was not allergic to drugs; *(iii)* the patient had no history of mental illness or of communication disorder. Pregnant and lactating women, severe joint malformations, loss of labor force, other lung diseases or lung infection, and severe diseases in the heart, liver, and kidney were the exclusion criteria. The ILD of the patients in the CTD-ILD group was diagnosed based on the 2000 American Thoracic Society (ATS)/ European Respiratory Society (ERS) criteria for idiopathic pulmonary interstitial fibrosis.[12] The subjects in the normal group were healthy according to physical examination results during the same period.

We collected fasting venous blood from all subjects. Some of the samples were submitted to the hospital laboratory for examination of serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), immunoglobulin (Ig) A, IgG, IgM, and complement (C) 3 and 4 levels. The remaining samples were separated from the serum after centrifugation at 3000 rpm for 10 min, and stored in a refrigerator at -80°C. In this section, Rayto rt-6000 automatic enzyme-linked immunosorbent assay (ELISA) machine (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, P.R.China) was used, to detect the expression levels of MMP-2, -3, -7, and -9 in the serum, and the kit was purchased from Elabscience company.

We used MasterScreen PFT (Jaeger GmbH, Hoechberg, Germany) to estimate lung function in the CTD-ILD group. Patients on long-acting beta-agonist and long-acting muscarinic receptor-agonist bronchodilators should be discontinued for at least 24 h, and patients on short-acting salbutamol bronchodilators should be discontinued for at least 6 h before testing. The detection indexes in this section include forced expiratory volume in one second (FEV1) and forced expiratory volume (FVC). After obtaining the data, we considered the FEV1% to

the predicted value and the ratio of FEV1 to FVC (FEV1/FVC). The data needed repeating more than three times to ensure an FEV1 error <5% using the mean result.

Statistical analysis

Data were analyzed with IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). The data were normally distributed and expressed as mean ± standard deviation (SD). A t-test was utilized for the comparison between two groups, and an F test was used for the comparison amid multiple groups. The categorical data were expressed as number and percentage, and the comparison between groups was performed by the chi-square test. Correlation analysis was carried out by the Pearson correlation test, GraphPad Prism version 5.0. (Graphpad Software Inc., San Diego, CA, USA) was used for plotting and analysis. A p value <0.05 was considered statistically significant.

RESULTS

The CTD-ILD group consisted of 47 male and 33 female patients, with a mean age of 48.1±12.5 years (range, 31 to 67 years). The CTD group consisted of 59 males and 41 females, with a mean age of 46.5±13.1 years (range, 29 to 65 years). The control group included 45 males and 35 females, with a mean age of 47.3±11.9 years (range, 30 to 65 years). In addition, there were no statistically significant differences in age, sex, and body mass index between the three groups (p>0.05).

By comparing the serum levels of MMP-2, -3, -7, and -9 in the three groups, we found significant differences among the data (p<0.05). Pair-to-pair comparisons of the data of the three groups showed that the MMP-2, -3, -7, and -9 levels in the serum of the CTD-ILD group were significantly higher than those in the CTD group and the control group (p<0.05). The MMP-2, -3, -7, and -9 levels in the serum of the CTD group were also significantly higher than those in the control group. General data and serum MMP levels of the three groups of subjects were summarized in Table 1.

The relevant analysis results of the serum levels of MMP-2, -3, -7, and -9 and the CRP, ESR, RF, IgA, IgG, and IgM of the patients in the CTD-ILD group were summarized in Table 2. The serum MMP-7 level was significantly positively correlated with CRP, ESR, and RF (p<0.05) but significantly negatively correlated with IgG and C3 (p<0.05). However, there was no significant correlation between serum MMP-2, -3, and -9 levels and CRP, ESR, RF, IgA, IgG, and IgM (p>0.05).

The correlation analysis results of the serum MMP-2, -3, -7, and -9 levels and the FEV1%,

Table 1. Comparison of basic data and serum MMP levels among the three groups

	The CTD-ILD (n=80)		The CTD (n=100)		The normal (n=80)		
Parameters	n	Mean±SD	n	Mean±SD	n	Mean±SD	*p*
Age (year)		48.1±12.5		46.5±13.1		47.3±11.9	0.193
Sex							0.272
Male	47		59		45		
Female	33		41		35		
BMI (kg/m^2)		23.2±2.7		23.4±2.6		23.1±2.5	0.216
MMP-2 (ng/mL)		11.97±2.37*#		26.87±17.23*		21.97±14.87	<0.05
MMP-3 (ng/mL)		299.08±41.00*#		103.55±93.56*		78.62±63.09	<0.05
MMP-7 (ng/mL)		17.90±4.13*#		12.57±7.59*		6.47±3.96	<0.001
MMP-9 (ng/mL)		57.93±7.45*#		42.83±16.28*		38.33±9.45	<0.05

SD: Standard deviation; MMP: Matrix metalloproteinases; CTD: Connective tissue disease; ILD: Interstitial lung disease; BMI: Body mass index; * Compared with the normal control group, the results of p<0.05 or # Compared with the CTD group, the results of p<0.05 were deemed significant statistically. The *p* values are of intergroup comparisons. Significance was accepted for *p* values <0.05.

Table 2. Correlation analysis between serum MMP levels and disease activity laboratory indexes in the CTD-ILD group (r)

Indicators	MMP-2	MMP-3	MMP-7	MMP-9
CRP	0.137	-0.082	0.526*	-0.122
ESR	0.055	0.099	0.794*	0.051
RF	0.167	-0.140	0.431*	-0.172
IgA	-0.065	-0.192	-0.259	-0.009
IgG	-0.029	-0.197	-0.301*	-0,030
IgM	0.029	0.220	-0.100	0.002
C3	0.011	-0.012	-0.310*	-0.113
C4	0.073	0.071	-0.118	-0.075

MMP: Matrix metalloproteinases; CTD: Connective tissue disease; ILD: Interstitial lung disease; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; RF: Rheumatoid factor; Ig: Immunoglobulin; C: Complement; It was accomplished through Pearson correlation analysis, and the parameter of significance was accepted if the *p* values was <0.05.

Table 3. Correlation analysis on the serum MMP levels and the disease activity clinical indicators in the CTD-ILD group (r)

Indicators	MMP2	MMP3	MMP7	MMP9
FEV1%	-0.063	0.163	0.621*	-0.123
FEV1/FVC	-0.266	-0.035	0.313*	-0.008
FVC	-0.013	0.120	0.547*	-0.054

MMP: Matrix metalloproteinases; CTD: Connective tissue disease; ILD: Interstitial lung disease; FEV1: Forced expiratory volume in one second; FVC: Forced expiratory volume; It was accomplished through Pearson correlation analysis, the value of significance was accepted if the *p* values was of <0.05.

FEV1/FVC, and FVC in CTD-ILD group patients were summarized in Table 3. Patients' serum MMP7 levels were significantly positively correlated with FEV1%, FEV1/FVC, and FVC

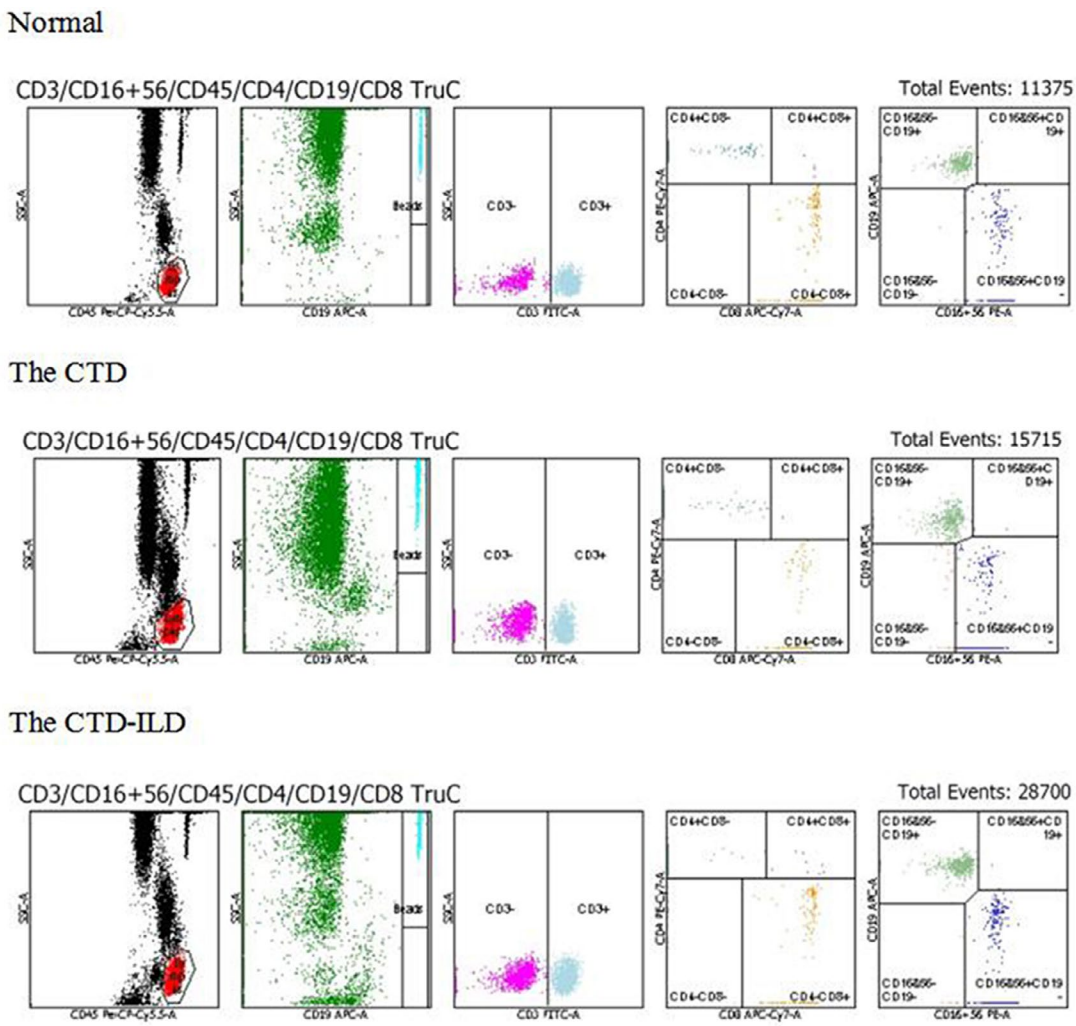

Figure 1. Distribution of T cell subsets in serum of three groups.

($p<0.05$), while serum MMP-2, -3, and -9 levels were not significantly correlated with FEV1%, FEV1/FVC, and FVC ($P>0.05$).

In normal, CTD, and CTD-ILD groups, the percentages of CD3+CD8+ cells were 22.92%, 11.25%, and 8.20%; the percentages of CD3+CD4+ cells were 45.55%, 47.65%, and 45.28%, respectively; the percentages of CD3+CD4+CD8+ cells were were 0.23%, 0.08%, and 0.32%; the percentages of CD16+CD56+ cells were 10.16%, 20.95%, and 19.62%, respectively; the percentages of CD19+ cells were 17.96%, 16.03%, and 18.66%, respectively (Figure 1, Table 4).

The correlation analysis results of the serum MMP-2, -3, -7, and -9 levels and the percentage of total lymphocytes, T cells, helper T cells, inhibitory T cells, B lymphocytes, natural killer cells, and the T helper/T suppressor ratio in the CTD-ILD group were summarized in Table 5. The expression level of MMP-7 in the serum was significantly positively correlated with the percentage of B cells ($p<0.05$), and there was no significant correlation between the serum MMP-2, -3, and -9 levels and the percentage of total lymphocytes, T cells, helper T cells, inhibitory T cells, B lymphocytes, natural killer cells, and the T helper/T suppressor ratio ($p>0.05$).

Table 4. Distribution of T cell subsets in the serum of three groups

Groups	Normal		CTD		CTD-ILD	
Parameters	Percent	Value/AbsCnt	Percent	Value/AbsCnt	Percent	Value/AbsCnt
Lymph events		1,732		2,401		2,487
Bead events		631		2,920		2,599
CD3+	71.07	1,860.55	61.52	482.40	61.28	559.23
CD3+CD8+	22.92	600.03	11.25	88.18	8.20	74.86
CD3+CD4+	45.55	1,192.50	47.65	373.64	45.28	413.18
CD3+CD4+CD8+	0.23	6.05	0.08	0.65	0.32	2.94
CD16+CD56+	10.16	266.01	20.95	164.28	19.62	179.07
CD19+	17.96	440.05	16.03	125.74	18.66	170.26
CD45+		2,617.76		784.19		912.60
4/8 Ratio		1.99		4.24		5.52

CTD: Connective tissue disease; ILD: Interstitial lung disease.

Table 5. Correlation analysis between serum MMP and lymphocyte levels in the CTD-ILD group (r)

Indicators	MMP-2	MMP-3	MMP-7	MMP-9
The total lymphocyte	-0.188	0.07	0.258	-0.046
The total T cell	-0.183	0.114	-0.346	0.273
Helper T cells	-0.399	0.145	-0.351	0.478
Inhibitory T cells	-0.02	0.096	0.102	0.071
B lymphocyte	-0.138	0.115	0.561*	0.096
NK cell	0.102	-0.212	-0.201	0.218
Th/Ts	0.637	-0.017	-0.290	-0.03

MMP: Matrix metalloproteinases; CTD: Connective tissue disease; ILD: Interstitial lung disease; NK: Natural killer; Th: T helper; Ts: T suppressor; It was conducted via Pearson correlation analysis, the outcome of significance was accepted when the P value was <0.05.

DISCUSSION

Connective tissue diseases are a common group of musculoskeletal system diseases, which often manifest as diffuse CTD and diseases of joints and soft tissues around joints. Connective tissue diseases are often chronic and repeated, aggravated by pulmonary interstitial lesions, which makes monitoring and timely treatment of disease activity a top priority.[13,14] Apart from the increasing levels of corresponding immune factors, CTD is characterized by inflammatory immune complex precipitation.[15]

In this study, we found that the serum levels of MMP-3, -7, and -9 in the CTD-ILD group and the CTD group were significantly higher than those in the control group, while the CTD-ILD group was also higher than the CTD group, suggesting that the elevated serum MMP level was an prominent feature of CTD and played a role in the occurrence and progression of the disease. The main function of MMPs is to degrade the components of the extracellular matrix and basement membrane composed of elastin, laminin, fibrin, and type 4 collagen.[16] It has been found that when the dynamic balance of synthesis and decomposition of extracellular matrix in the lung is disrupted, the imbalance of MMPs and MMP tissue inhibitors will lead to the abnormal increase of the extracellular matrix, resulting in pulmonary fibrosis and alveolar structure reconstruction, which may be the reason for the further increase of the serum MMP level in patients with ILD.[17]

Of the members of the MMP family, MMP-3 is one of the most critical proteases in the process of cartilage degradation in osteoarthritis and rheumatoid arthritis. It is mainly secreted by chondrocytes, macrophages, and fibroblasts, and its expression is regulated by a variety of cytokines and growth factors, including induction factors such as tumor necrosis factor-alpha (α), interleukin-6, and interferon-α and inhibitory factors such as interleukin-4 and transforming growth factor-beta (β).[18] In CTDs, plasminase, cathepsin G, and other factors could remove the MMP-3 proenzyme peptide to form biologically active MMP-3. The activated MMP-3 further excises the MMP-3 proenzyme peptide and activates it to produce a cascade amplification effect, resulting in a notable surge in serum MMP-3 levels.[19] Ma et al.[20] used a receiver operating characteristic curve to analyze the application value of MMP-3 in the evaluation of rheumatoid arthritis disease activity and found that the sensitivity of MMP-3 combined with CRP was 97.2%, which was significantly higher than that of 84.7% using CRP alone. These results indicated that MMP3 plays an important role in assessing the activity of CTD.

We conducted a correlation analysis and found that the serum MMP-7 levels of the CTD group and the CTD-ILD group were significantly positively correlated with CRP, ESR, RF, FEV1%, FEV1/FVC, and FVC ($p<0.05$) and significantly negatively correlated with IgG and C3 ($p<0.05$); however, there was no significant correlation between MMP-2, -3, and -9 and these activity indicators ($p>0.05$), suggesting that serum MMP-7 could be used as an indicator of the disease activity of CTD complicated with ILD. In this study, we also found that there was a significant positive correlation between the serum MMP-7 level and the percentage of B lymphocytes in the CTD-ILD group ($p<0.05$), and thus we had reason to speculate that the increased expression of MMP-7 might be related to the activation of B lymphocytes and cytokine secretion. Subsequent experiments will be conducted to further verify this experimental conclusion. MMP-7 is a major protease that degrades fibrin, type 4 collagen, elastin, laminin, and other important components of the extracellular matrix and basement membrane. When the dynamic balance between the synthesis and degradation of the extracellular matrix in the lung is disrupted, the imbalance between the extracellular matrix and MMPs or the imbalance between MMPs and tissue inhibitors of MMPs leads to the abnormal increase of the extracellular matrix, resulting in pulmonary fibrosis and alveolar reconstruction.[21] Our study found that the significant positive correlation between MMP-7 and lung function indicators suggests the involvement of MMP-7 in interstitial lung lesions, and the significant correlation between MMP-7 and immune-related indicators also indicated that MMP-7 was also involved in immune responses.

The shortcomings of this study are as follows: this study only detected MMPs in the serum and failed to detect mononuclear cells or other tissues in blood; studies of over-expression or silencing of MMPs on cell or animal level were not carried out

to further elaborate the function and mechanism of MMPs in CTD with interstitial lung lesions. The above deficiencies will be improved in a follow-up study.

In conclusion, we preliminarily confirmed that serum levels of MMP-3, -7, and -9 were significantly increased in CTD patients with interstitial lung lesions, whereas high levels of MMP-7 indicated active interstitial lung lesions, which could serve as an essential marker for early diagnosis of ILD and evaluation of disease progression. The unexplained role of MMP-7 in interstitial lung lesions will be further explored in subsequent studies.

Ethics Committee Approval: This study was approved by the committee of Affiliated Hospital of Youjiang Medical University for Nationalities Ethics Committee (date: 15.03.2022, no: Z20200163). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, control/supervision, analysis and interpretation, writing the article, references and fundings: H.C., L.T.; Design: J.T., L.T.; Data collection and processing: J.L., D.H., C.P., S.L., X.D.; Literature review, critical review, materials: H.C., J.T., J.L., D.H., C.P., S.L., X.D., L.T.

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