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Changes and clinical significance of serum MMP-9, TIMP-1, COX-2, and immune levels in patients with asthma

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Abstract

Objective: To detect serum metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinases (TIMP-1), cyclooxygenase-2 (COX-2), and T helper cells 1-T helper cells 2 (Th1-Th2) levels in asthma patients and assess their clinical significance.

Methods: A total of 72 patients experiencing acute asthma (acute group), 66 stable asthma patients (stable group), and 60 healthy volunteers (control group) were included in this study. The levels of TIMP-1, COX-2, and Th1-Th2 in patients with acute asthma were measured following treatment with budesonide aerosol inhalation. In addition, the levels of MMP-9, TIMP-1, COX-2 and Th1-Th2 were compared in patients with different severity of acute asthma before and after treatment.

Results: The serum levels of MMP-9, TIMP-1, and COX-2 showed an increasing trend in the control, stable, and acute groups, while levels of Th1-Th2 showed a sequential decreasing trend, and the differences were statistically significant. Comparison of lung function indexes among the three groups of patients established a negative correlation between serum MMP-9 and its forced vital capacity% predicted (FEV₁%pred), TIMP-1, and COX-2, and FEV₁%pred and forced expiratory volume in 1 s-forced vital capacity (FEV₁/FVC) levels, but a positive correlation between Th1-Th2 and FEV₁/FVC levels in the acute group. A significant difference was observed on comparing the levels of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 in patients with different conditions in the acute group. Specifically, as the condition worsened, a significant increase in serum MMP-9, TIMP-1, and COX-2 levels but a significant decrease in Th1-Th2 levels was observed. After treatment, we observed a significant decrease in serum MMP-9, TIMP-1, and COX-2 levels but a significant increase in Th1-Th2 levels in the acute group.

Conclusion: The serum levels of MMP-9, TIMP-1, COX-2, and Th1-Th2 are valuable indicators reflecting the condition of asthma patients and could be considered promising clinical monitoring indicators.

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Introduction

Airway hyperresponsiveness and persistent airway inflammation are two key characteristics of asthma, a diverse disease.¹ In China, the prevalence of asthma in adults is currently 1.24%, and the disease carries a comparable risk of death and disability to type 2 diabetes mellitus (T2DM).^{2,3} Although standardized asthma therapy has been aggressively promoted in clinical settings, the clinical control proportion of asthma still falls short of the desired level, resulting in China having one of the highest asthma fatality proportions globally.⁴⁻⁶ Therefore, actively monitoring of asthma patients using effective means is crucial for improving their prognosis and extending their lifespan. It has been established that airway remodeling plays a central role in the pathogenesis of asthma and is a significant component in the early stages of the disease.^{7,8} The severity of airway remodeling is closely associated with the severity of asthma. Matrix metalloproteinase-9 (MMP-9) is a crucial enzyme involved in airway remodeling, while tissue inhibitor of metalloproteinase-1 (TIMP-1) is a natural endogenous inhibitor of MMPs responsible for inhibiting extracellular matrix (ECM) degradation.⁹ Cyclooxygenase (COX) is an important rate-limiting enzyme in prostaglandin synthesis and one of its forms, cyclooxygenase-2 (COX-2), is an inducible enzyme that exerts an “inflammatory response.”¹⁰ A balance between T helper cell subsets 1 and 2 (Th1-Th2) is used as an indicator of the patient’s immune function. Studies have reported that asthma patients often experience disruption in the Th1-Th2 balance, which is also implicated in the onset and progression of asthma.^{11,12} Given these factors, we conducted the present study to investigate the clinical value of serum levels of MMP-9, TIMP-1, COX-2, and Th1-Th2 in reflecting the condition of asthma patients.

Material and Methods

Study subjects

This study comprised 138 asthma patients admitted to our hospital between August 2019 and August 2021 and a control group comprising 60 healthy individuals with no personal or hereditary history of allergy.

- (1) **Inclusion criteria:** Patients aged 18-80 years, and satisfied the relevant diagnostic criteria of the Guidelines for the Prevention and Treatment of Bronchial Asthma established by the Chinese Medical Association.¹³ The patients that were not treated with oral hormone or immunosuppressive therapy within 4 days prior to participation in the study were also included in the study.
- (2) **Exclusion criteria:** Patients with acute infections in organs other than the respiratory system or with significant organ lesions, and with presence of other autoimmune diseases were excluded from the study. Women who were pregnant or lactating were also excluded from the study.

Based on the diagnostic criteria, clinical staging, and the severity classification outlined in the Diagnostic Criteria,

Clinical Staging and Severity Classification of Bronchial Asthma,¹⁴ patients with acute exacerbation of asthma were divided into the acute group ($n = 72$), while 66 patients with stable asthma were included in the stable group ($n = 66$). The acute group comprised 40 males and 32 females aged 24-78 years, with a mean age of 59.45 ± 10.15 years. The stable group consisted of 36 males and 30 females, aged 22-80 years, with a mean age of 61.15 ± 11.02 years. The control group, comprising individuals in good health, had 35 males and 25 females aged 23-78 years, with a mean age of 60.44 ± 10.45 years. Statistical analysis showed no significant differences in gender and age among the three groups ($P > 0.05$). Ethical approval for this study was obtained from the Ethics Committee of The Second Affiliated Hospital of Wannan Medical College. Written informed consent was obtained from all subjects and a legally authorized representative(s) for anonymized patient information to be published in this article.

Methods

Testing of indicators

(1) Detection of serum MMP-9, TIMP-1, and COX-2 levels

A total of 8 mL of peripheral fasting venous blood was drawn from each individual and collected in anticoagulation tubes containing sodium heparin. The tubes were left at room temperature for 30 min, then centrifuged at 500 rpm for 10 min using a centrifuge with a radius of 10 cm. The resulting supernatant was carefully collected and stored in a freezer at -80°C until further use. The serum MMP-9, TIMP-1, and COX-2 levels were determined using a double antibody sandwich enzyme-linked immunosorbent serologic assay (ELISA). The MMP-9 and TIMP-1 test kits were obtained from Wuhan Eli Reiter Technology Co. Ltd., while the COX-2 test kit was purchased from Cayman Company (Spain). The ELISA procedures were strictly performed according to the provided instructions and applicable regulations.

(2) Th-subpopulation assay

Flow cytometry was used to measure peripheral blood Th1 and Th2 levels using a kit purchased from BD (USA), specifically the BD multitest IMK 4-color lymphocyte subpopulation kit. Two tubes, a negative control tube (tube A) and an assay tube (tube B), were prepared. In both tubes, 100 μL of blood and 100 μL of LPM I 1640 were added. Then, 4 μL of Brefeldin A (BFA) working solution was added to tube A, while to tube B, 5 μL of phorbol 12-myristate 13-acetate (PMA) working solution was added. Both tubes were then incubated at 37° in a 5% CO incubator for 4 h. Next, solutions of tubes A and B were blended. Then, 20 μL of CD3PerCP and 5 μL of cluster of differentiation 8 allophycocyanin (CD8-APC) were added to the mixture and incubated for 15 min at room temperature, away from light.

The mixture was divided into four tubes, labeled A1, A2, B1, and B2. To each of these tubes, 100 μL of the previously stained whole blood was added. Then, 1 mL of

1× fluorescence-activated cell sorting (FACS) hemolysin was added to each tube, thoroughly mixed, and incubated for 10 min at room temperature, away from light. Afterward, 2 mL of phosphate-buffered saline (PBS) solution was added, mixed well, and centrifuged at 2500 rpm for 5 min. After discarding the supernatant, 0.5 mL of 1× FACS permeation solution was added to each tube, mixed well, and incubated for 10 min at room temperature, away from light. Following this, 2 mL of PBS solution was added, mixed well, and centrifuged at 2500 rpm for 5 min. After discarding the supernatant, 20 µL of IFN γ -FITC/IL-4 PE was added to tubes A1 and B1, while 20 µL of isotype control r2a-FITC/r1-PE was added to tubes A2 and B2. The contents in the tubes were mixed well, and the tubes were incubated for 30 min at room temperature, away from light. Then, the tubes were washed once with PBS, the supernatant was removed, and the cell precipitate was resuspended in 200 mL of poly(ether sulfone) (PES). The samples were analyzed using a flow cytometer with its corresponding software. A control was set using CD3 to select lymphocytes, and the identified lymphocytes were analyzed using CD8, interleukin 4 (IL-4), and interferon gamma (IFN- γ) to determine proportion of Th2 and Th1 cells. The Th1-Th2 ratio was calculated based on the analysis.

Observed indicators

- (1) Serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels were compared in patients in acute, stable, and control groups.
- (2) The investigated patients' FEV $_1$ % and FEV $_1$ -FVC levels were measured using the MedGraphics 1085D lung function meter.
- (3) The relationship between pulmonary function measures and serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels in individuals in the acute phase of asthma was examined using Pearson linear correlation analysis.
- (4) Patients in the acute group were classified according to the criteria for assessing the severity of acute asthma exacerbations provided in the 2018 Chinese Expert Consensus on the Assessment and Management of Acute Bronchial Asthma Exacerbations.¹⁵ The patients were divided into a mild, moderate or critical sub-groups. Then, the serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels of patients with different conditions were compared in the acute phase.
- (5) Peripheral venous blood was collected from patients before and after treatment in the acute phase to

measure and compare serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels.

Statistical Methods

The SPSS v19.0 statistical software was used to process the data. The data are expressed as ($\bar{x} \pm s$), and one-way ANOVA was used to compare multiple groups to test for significance. Fisher's least significant difference (LSD)-*t* test was used for two-way comparison, and paired sample *t*-test was used for mean data before and after treatment. Count data are expressed as *n* (%), and the χ^2 test was used to compare groups. Pearson's linear correlation was performed for correlation analysis, and differences were considered statistically significant at *P* < 0.05.

Results

Comparison of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels in three groups

The serum MMP-9, TIMP-1, and COX-2 levels in the control, stable, and acute groups showed an increasing trend, and Th1-Th2 showed a sequential decreasing trend, with the differences being statistically significant between two groups (*P* < 0.05) (Table 1).

Comparison of pulmonary function indexes in three groups

The differences were statistically significant (*P* < 0.05), compared to the lung function indexes of the three groups (Table 2).

Table 2 Comparison of pulmonary function indexes in three groups.

Group	N	FEV%pred	FEV $_1$ /FVC (%)
Control group	60	98.79±10.15	83.55±11.35
Stable group	66	90.15±9.88	76.71±9.43
Acute group	72	69.56±7.65	66.48±8.76
<i>F</i>		172.548	49.699
<i>P</i>		<0.001	<0.001

FEV%pred: forced vital capacity% predicted; FEV $_1$ /FVC: forced expiratory volume in 1 s-forced vital capacity.

Table 1 Comparison of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels in three groups

Group	n	MMP-9 (ng/mL)	TIMP-1 (ng/mL)	COX-2 (ng/mL)	Th1 (%)	Th2 (%)	Th1-Th2
Control group	60	34.48±7.45	81.45±13.15	7.45±1.16	16.16±2.16	15.63±2.15	1.03±0.21
Stable group	66	187.45±20.36	98.45±16.52	16.37±3.15	14.53±2.47	17.20±3.14	0.84±0.19
Acute group	72	347.91±36.58	144.15±22.67	40.55±6.89	12.15±2.18	17.15±3.17	0.71±0.15
<i>F</i>		2694.067	217.252	990.199	51.968	6.437	50.011
<i>P</i>		<0.001	<0.001	<0.001	<0.001	0.002	<0.001

Note: Compared to the control group, ^a*P* < 0.05 and ^b*P* < 0.05.

Correlation analysis between serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels and pulmonary function indices in patients of acute group

Correlation analysis suggested a negative correlation between serum MMP-9 and its forced vital capacity% predicted (FEV%pred), TIMP-1 and COX-2 with FEV%pred and forced expiratory volume in 1 s-forced vital capacity (FEV₁/FVC) levels, and a positive correlation between Th1-Th2 and FEV₁/FVC in the acute group of patients (Table 3).

Comparison of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels in patients with different conditions in the acute group

The differences in serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels were found to be statistically significant ($P < 0.05$) while comparing patients with different conditions within the acute group. Moreover, as the conditions worsened, a sequential increase in serum MMP-9, TIMP-1, and COX-2 levels, and a decrease in Th1-Th2 levels, was observed. These differences were also statistically significant when comparing the two groups with each other ($P < 0.05$) (Table 4).

Table 3 Correlation analysis between serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels and pulmonary function indexes in the acute group of patients.

Indicators	FEV%pred		FEV ₁ /FVC (%)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
MMP-9	-0.366	<0.001	-0.221	>0.05
TIMP-1	-0.267	<0.05	-0.311	<0.001
COX-2	-0.376	<0.001	-0.325	<0.001
Th1-Th2	0.178	>0.05	0.278	<0.05

Comparison of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels before and after treatment in acute group

It was observed that serum levels of MMP-9, TIMP-1, and COX-2 decreased, while that of Th1-Th2 increased significantly after treatment, compared to the levels before treatment, in the acute group of patients ($P < 0.05$) (Table 5).

Discussion

The treatment of bronchial asthma primarily relies on bronchodilators, including direct-acting bronchodilators, such as receptor agonists like salbutamol, birtolol, and levosadine, as well as indirect-acting bronchodilators, such as anti-cholinergics. Currently, asthma diagnosis that lacks specificity is mainly based on medical history and physical examination, and there is a lack of reliable markers for monitoring of the disease. Therefore, it is crucial to identify effective serum markers for prevention and treatment of asthma, starting by understanding its underlying pathogenesis. In this study, we investigated serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels in patients with stable asthma, acute asthma, and varying severity stages of acute asthma, both before and after treatment. Our findings showed differences in these markers among different asthma groups and demonstrated changes following treatment, suggesting that these markers have potential value in monitoring progression of asthma. Airway remodeling is a significant aspect of asthma pathogenesis with its key structural changes, including ECM deposition, epithelial shedding and proliferation, and vascular formation, among which ECM deposition in the airway wall may lead to fibrosis of the airway wall and airflow limitation.^{16,17} MMPs are a class of highly conserved zinc- and calcium-dependent protein hydrolases involved in various physiological activities,

Table 4 Comparison of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels in patients with different acute asthma severity.

Group	N	MMP-9 (ng/mL)	TIMP-1 (ng/mL)	COX-2 (ng/mL)	Th1-Th2
Mild group	30	203.15±22.13	119.26±11.59	30.24±7.45	0.81±0.21
Moderate group	20	375.48±34.87 ^a	137.48±16.58 ^a	42.02±8.66 ^a	0.72±0.18 ^a
Severe group	19	611.15±62.58 ^{a,b}	184.15±30.11 ^{a,b}	53.26±9.48 ^{a,b}	0.59±0.19 ^{a,b}
Critical	3	726.15±71.45 ^{a,b,c}	199.47±33.41 ^{a,b,c}	65.15±10.78 ^{a,b,c}	0.53±0.17 ^{a,b,c}
<i>F</i>		361.454	41.546	46.853	7.933
<i>P</i>		<0.001	<0.001	<0.001	<0.001

Note: Comparison to the mild group, ^a $P < 0.05$; comparison to the moderate group, ^b $P < 0.05$; and comparison to the severe group, ^c $P < 0.05$.

Table 5 Comparison of serum MMP-9, TIMP-1, COX-2, and Th1-Th2 levels before and after treatment in the acute group of patients.

Group	N	MMP-9 (ng/mL)	TIMP-1 (ng/mL)	COX-2 (ng/mL)	Th1-Th2
Before treatment	72	347.91±36.59	144.15±16.38	40.55±8.45	0.71±0.16
After treatment	72	221.69±20.69	113.69±11.99	20.69±7.76	0.87±0.21
<i>t</i>		37.396	18.221	20.792	-7.339
<i>P</i>		<0.001	<0.001	<0.001	<0.001

including inflammatory responses, malignant infiltration, and atherosclerosis, and are major rate-limiting enzymes in the regulation of ECM.^{18,19} Among them, MMP-9 belongs to type IV collagenase, which participates in the degradation and reconstruction of ECM, regulates cell adhesion, and directly or indirectly participates in the remodeling of tissue models and trauma repair.²⁰ Under normal conditions, MMP-9 is minimally expressed in the epivascular wall, but under inflammatory conditions, activation of various inflammatory cells promotes high MMP-9 expression, leading to excessive ECM degradation and destruction of the basement membrane.²¹

Inhibitors of metalloproteinase are a natural endogenous inhibitor of MMPs that also inhibit the degradation of ECM, resulting in collagen deposition; among TIMPs, TIMP-1 specifically inhibits MMP-9.^{22,23} In this study, we found that levels of serum MMP-9 and TIMP-1 were higher in patients with asthma than in controls, and levels of serum MMP-9 and TIMP-1 were higher in patients with acute asthma than in patients with stable asthma. The serum levels of MMP-9 and TIMP-1 in patients with acute asthma were higher before treatment. After treatment, the serum levels of MMP-9 and TIMP-1 in patients with acute asthma decreased significantly. Overall, these results suggest that serum MMP-9 and TIMP-1 may reflect changes in asthma levels.

COX-2, one of the isoforms of COX, has been found to be abnormally elevated in the airway epithelial cells and inflammatory cells of asthma patients.^{24,25} Normally, COX-2 is not expressed in the airway epithelial and smooth muscle cells under physiological conditions but is activated and expressed in response to inflammatory factors. In the airway mucosa of asthma patients, a significant infiltration of monocytes, macrophages, eosinophils, and mast cells has been reported to release substantial amount of COX-2, triggering acute asthma attacks or exacerbating asthma symptoms.^{26,27}

The present study observed that serum levels of COX-2 were significantly higher in patients with acute asthma, compared to the stable asthma group. Furthermore, there was a positive correlation between the severity of the acute stage and the serum levels of COX-2. Importantly, after treatment, the study reported a significant decrease in serum COX-2 levels in patients with acute asthma, suggesting that serum COX-2 level is a valuable marker for monitoring asthma patients and assessing treatment efficacy.

Th1-Th2 balance is crucial for maintaining normal immune function in the body. An imbalance between Th1 and Th2 responses disrupt immune homeostasis, leading to altered immune responses and the development of various diseases.²⁸ Th1 cells primarily secrete IL-2, IL-12, and IFN- γ , which activate macrophages and induce cellular immunity. They are also an important effector in type IV metaplasia. Comparatively, Th2 cells mainly secrete IL-4, IL-9, and IL-10, which specifically react with B cells, mast cells, and eosinophils and cause asthma inflammation.^{29,30} In this study, asthma patients exhibited lower Th1-Th2 levels than healthy controls. Moreover, the Th1-Th2 ratio was even lower in patients with acute asthma, compared to the stable asthma group, and the severity of the acute attack was associated with a further reduction in this ratio. However, after treatment, there was an improvement in Th1-Th2

level in patients with acute asthma. Collectively, Th1- Th2 cells could be useful in assessing asthmatic patients.

In addition, this study observed that lung function indexes, such as FEV₁%pred and FEV₁/FVC levels, were significantly decreased in asthma patients, compared to healthy controls. The decline in lung function indexes was more pronounced in patients experiencing the acute phase of asthma, indicating that asthma significantly impaired lung function. Correlation analysis revealed a negative correlation between serum MMP-9 levels and FEV₁%pred as well as between TIMP-1 and COX-2 levels with FEV₁%pred and FEV₁/FVC levels. A positive correlation was also observed between Th1-Th2 levels and FEV₁/FVC. These findings suggest that serum markers can influence lung function and contribute to the severity of asthma.

Conclusion

The serum levels of MMP-9, TIMP-1, COX-2, and Th1-Th2 are valuable indicators reflecting the condition of asthma patients and show promise as potential indicators for clinical monitoring. However, it is important to note that the sample size in this study was limited, which may introduce bias to the obtained conclusions. To enhance the reliability of the findings, the future studies must consider conducting multicenter research with larger sample sizes.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors stated that there was no conflict of interest to declare.

Author Contributions

Hong Ming and Youming Huang designed the study, completed the experiment, and supervised the data collection. Jinjuan Mao analyzed and interpreted the data. Hui Wang, Xiufeng Gao, and Zhidian Li prepared the manuscript for publication, and reviewed its draft. All authors read and approved the final manuscript.

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