EXPRESSION AND CORRELATION OF SERUM HMGB1, NE, AND IL-1B IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Objective: *To analyze the expression level of serum high mobility group protein 1 (HMGB1), neutrophil elastase (NE), interleukin-1 β (IL-1β) in patients with chronic obstructive pulmonary disease (COPD) and its correlation with pulmonary function.*

Methods: *We selected 118 COPD patients who were admitted to the respiratory department of our hospital from April 2018 to November 2019 as the study objects. According to the severity of the disease, they were divided into an acute exacerbation group (58 cases) and a stable group (60 cases). In addition, 40 healthy people who had a physical examination in our hospital at the same time were selected as the normal control group. The expression levels of HMGB1, NE, and IL-1β in serum from all the subjects were detected by ELISA and a pulmonary function instrument. The ratio of forced expiratory volume to forced vital capacity (FEV1/FVC) and FEV1% PRED in the first second was measured. Pearson analysis was used for correlation analysis.*

Results: *The serum levels of HMGB1, NE, and IL-1β in the acute exacerbation group and the stable group were significantly higher than those in the normal control group (P<0.05). The serum levels of HMGB1, NE, and IL-1β in the acute exacerbation group were significantly higher than those in the stable group (P<0.05). FEV1% PRED and FEV1/FVC in the acute exacerbation group and the stable group were significantly lower than those in the normal control group (P<0.05). FEV1% PRED and FEV1/FVC in the acute exacerbation group were significantly lower than those in the stable group (P<0.05). Serum HMGB1, NE, and IL-1β were negatively correlated with pulmonary function indexes in patients with acute exacerbation, and, in the stable stage, serum HMGB1 was positively correlated with NE and IL-1β, and NE was positively correlated with IL-1β (P<0.05).*

Conclusion: *The expression levels of HMGB1, NE, and IL-1β in the serum of COPD patients were significantly increased and were negatively correlated with FEV1/FVC and FEV1% PRED. The three indexes can reflect the severity of COPD patients to a certain extent, which is worthy of clinical application.*

Keywords: *Chronic obstructive pulmonary disease, HMGB1, NE, IL-1β, pulmonary function, correlation.*

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Introduction

Chronic obstructive pulmonary disease (COPD) is a persistent chronic inflammatory disease, characterized by continuous airflow restriction. The airflow restriction is a progressive development, accompanied by the airway and lung chronic inflammatory response to harmful particles and gases. Both morbidity and mortality rates are high. COPD not only involves the lung but also can cause diabetes, cardiovascular disease, anxiety and depression, metabolic syndrome, obstructive sleep apnea, and other complications that seriously affect human health and quality of life[1]. At present, the pathogenesis of COPD is still not completely clear, and it is generally believed to be related to inflammatory mediators, oxidative stress, protease-antiprotease imbalance mechanism,

etc., and to be a systemic multi-system chronic inflammatory disease mediated by multiple factors and biological mechanisms[2]. Clinical diagnosis of COPD still depends on laboratory and imaging examinations, but it is prone to misdiagnosis due to individual differences among patients and other factors. Therefore, it is of great importance to search for specific biological indicators to improve the diagnosis of COPD and the prognosis of patients.

Serum high mobility group protein 1 (HMGB1) is a non-histone protein with a variety of extranuclear biological functions and participates in the pathological process of a variety of inflammatory diseases by mediating inflammatory responses[3].

Neutrophil elastase (NE) is an important member of the serine protease family that can promote the release of various pro-inflammatory cytokines in epithelial cells and aggravate the inflammatory response[4].

Interleukin 1β (IL-1β) is an important pro-inflammatory cytokine that can cause pulmonary inflammation characterized by granulocyte and macrophage infiltration in the lung[5]. This study aimed to analyze the expression levels and correlation of HMGB1, NE, and IL-1β in the serum of COPD patients.

Materials and methods

General information

A total of 118 patients with COPD admitted to the Department of Respiratory Medicine of our hospital from April 2018 to November 2019 were selected as the study subjects. According to the severity of the disease, they were divided into an acute exacerbation group (n = 58) and a stable group (n = 60).

Inclusion criteria were as follows:

• All patients met the diagnostic criteria for COPD in the Guidelines for the Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease[6];

• All patients were 50 to 80 years old;

• Stable COPD patients were stable for more than 3 months with the same treatment plan and no lower respiratory tract infection found on their chest radiograph;

• Patients had no recent use of glucocorticoids;

• There was complete clinical case data.

This study was approved by the ethics committee of the hospital, and all patients and their families agreed to participate in this study.

Exclusion criteria were established as follows:

• Patients with lung cancer, asthma, active pulmonary tuberculosis, and other lung diseases;

• Patients whose acute exacerbation lasted more than 5 d;

• Patients who had been treated with antibiotics within 1 month before enrollment;

• Patients with cardiovascular and cerebrovascular diseases and mental disorders who could not complete the test;

• Patients with a positive bronchial dilation test;

• Patients with blood system diseases or connective tissue diseases.

In the acute exacerbation group, there were 41 males and 17 females, aged from 50 to 80 years, with an average of (61.02±7.14) years, BMI of (23.02±2.87) kg/m^2, and COPD duration of (8.64±3.52) years. In the stable group, there were 42 males and 18 females, aged from 50 to 80 years, with a mean age of (60.47±6.23) years, BMI of (24.36±3.42) kg/m^2, and COPD duration of (7.81±3.33) years. In addition, 40 healthy people who came to our hospital for a physical examination during the same period were selected as the normal control group, including 24 males and 16 females, aged from 50 to 80 years old, with an average age of (59.03±5.47) years old and a BMI of (23.65±3.48) kg/m^2. A statistical test showed that there was no statistical significance in the general data of the three groups (P>0.05).

Methods

Serum HMGB1, NE, IL-1β levels: Fasting venous blood (6 ml) was collected from all the patients on the second day after admission. The normal control group had venous blood taken during a physical examination, which was placed at room temperature for 30 min and then centrifuged at 3000 r/min with a centrifugation radius of 8 cm for 10 min. The serum was separated and transferred to a 1 ml clean EP tube, which was stored in a refrigerator at –80°C for later use. Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of HMGB1, NE, and IL-1β in the serum of all subjects. Serum HMGB1 kit was provided by Wuhan Vexeth Technology Co., Ltd., the NE kit was provided by Wuhan Feien Biotechnology Co., Ltd., and the IL-1β kit was provided by Beijing Luyuan Bird Biological Technology Co., Ltd.

Lung function test

All the research object with German jaeger company production of lung function tests. Pulmonary

function testing measured lung function in patients who were to stop using a long-acting bronchodilator and anticholinergic drugs for at least 24 h. After routine pulmonary function testing, application dose control subjects inhaled albuterol inhalers to 400 μg. After a rest of 15 min, the forced expiratory volume in the first second (FEV1)/forced vital capacity (FVC) and FEV1 percentage of predicted FEV1 (FEV1% PRED) was examined again. Each index was measured twice, and the best value was obtained when FEV1/FVC <70% indicated limited airflow. All operations are conducted in strict accordance with the instructions.

Statistical methods

The data in this study were analyzed by the SPSS21.0 software package, and the measurement data were expressed as ($\bar{x}\pm s$). The comparison of data between two groups was tested by t-test, and the comparison of data between multiple groups was made by ANOVA. All count data were expressed as (n[%]), and the comparison of data between two groups was tested by χ^2. The correlation between serum HMGB1, NE, and IL-1β with pulmonary function indexes was analyzed by Pearson analysis. P<0.05 was considered statistically significant.

Results

Comparison of serum levels of HMGB1, NE, and IL-1β in each group

Serum levels of HMGB1, NE, and IL-1β in the acute exacerbation group and the stable group were significantly higher than those in the normal control group, and the differences were statistically significant (P < 0.05). The levels of serum HMGB1, NE, and IL-1β in the acute exacerbation group were significantly higher than those in the stable group, and the differences were statistically significant (P<0.05). See Table 1.

Group	N	HMGB1 (ng/L)	NE (μg/L)	IL-1β (ng/L)
Acute exacerbation group	58	8.28±2.02*#	100.82±18.61*#	25.46±3.74*#
Stable group	60	5.46±1.33*	77.54±11.46*	14.26±5.32*
Normal control group	40	2.19±0.83	58.79±10.15	6.44±2.89
F		187.92	106.30	249.85
P		<0.001	<0.001	<0.001

Table 1: Comparison of serum levels of HMGB1, NE, and IL-1β in each group ($\bar{x}\pm s$).
*Note: Compared with the normal control group, *P<0.05; compared to the stable group, #P<0.05.*

Comparison of pulmonary function indexes in each group

The pulmonary function indexes FEV1% PRED and FEV1/FVC in the acute exacerbation group and the stable group were significantly lower than those in the normal control group, and the differences were statistically significant (P<0.05). The FEV1% PRED and FEV1/FVC ratio in the acute exacerbation group were significantly lower than those in the stable group, and the differences were statistically significant (P<0.05). See Table 2.

Group	N	FEV1% PRED	FEV1/FVC
Acute exacerbation group	58	25.16±4.78*#	57.57±4.86*#
Stable group	60	36.42±5.21*	65.81±3.78*
Normal control group	40	66.41±5.89	78.63±4.29
F		754.44	280.04
P		<0.001	<0.001

Table 2: Comparison of lung function indexes in each group ($\bar{x}\pm s$).
*Note: Compared with the normal control group, *P<0.05; compared to the stable group, #P<0.05.*

Correlation between serum C and pulmonary function indexes in patients with acute exacerbation

Serum HMGB1, NE, and IL-1β were significantly negatively correlated with pulmonary function indexes in patients with acute exacerbation. Serum HMGB1 level was significantly positively correlated with the levels of NE and IL-1β, and NE was significantly positively correlated with IL-1β (P<0.05). See Table 3.

	HMGB1		NE		IL-1β	
	r	*P*	r	*P*	r	*P*
FEV1% PRED	–0.669	0.003	–0.489	<0.001	–0.823	<0.001
FEV1/FVC	–0.717	0.015	–0.363	0.002	–0.790	<0.001
NE	0.663	<0.001	-	-	-	-
IL-1β	0.625	0.004	0.537	<0.001	-	-

Table 3: Correlation between serum HMGB1, NE, and IL-1β and pulmonary function indexes in patients with acute exacerbation.

Correlation between serum HMGB1, NE, and IL-1β and pulmonary function indexes in patients with stable stage

Serum HMGB1, NE, and IL-1β were significantly negatively correlated with pulmonary function indexes in stable stage patients. Serum

HMGB1 level was significantly positively correlated with the levels of NE and IL-1β, and NE was significantly positively correlated with IL-1β (P<0.05). See Table 4.

	HMGB1		NE		IL-1β	
	r	*P*	r	*P*	r	*P*
FEV1% PRED	–0.362	0.002	–0.316	0.012	–0.503	0.009
FEV1/FVC	–0.489	<0.001	–0.215	<0.001	–0.526	0.004
NE	0.293	0.037	-	-	-	-
IL-1β	0.303	0.031	0.131	0.024	-	-

Table 4: Correlation between serum HMGB1, NE, and IL-1β and pulmonary function indexes in patients with stable stage.

Discussion

COPD for a variety of inflammatory cells and cytokines involved in chronic inflammatory diseases and inflammation in the lungs is closely related to the deterioration of lung function. Inflammatory infections cause inflammation cells on the surface of the central airway epithelium, induced by upper respiratory tract mucous membrane bleeding. As mucus secretion increases with the lung ventilation function of patients with influence, mucous gland enlargement leads to repeated airway wall injury and repair processes[7]. Repeated infection and hypoxia in COPD patients are important factors that exacerbate the disease and are also the main reasons for patients to seek medical treatment. At present, the clinical treatment of COPD is mainly to control infection as soon as possible, relieve symptoms, and prevent and control the frequency of acute attacks, but there is still a lack of relevant, effective, objective evaluation indicators. Relevant data have shown that multi-target and multi-channel can block the inflammatory pathway of COPD, and further study of the cytokines in the inflammatory pathway is needed to determine how to effectively block the natural course of COPD[8].

Late-stage HMGB1 is an important inflammatory mediator due to its small molecular weight and a greater ability to migrate in polyacrylamide gel electrophoresis. It is widely distributed in the lymphoid tissue, brain, liver, lungs, and heart tissue cells, and, in biological functions, it has a variety of extranuclear in acute lung injury, hypersensitivity pneumonitis, and other high expressions in lung diseases. In addition to its nuclear function, HMGB1 is released later than other inflammatory mediators such as TNF and IL-1 in the course of the disease and is considered to be an important late-stage inflammatory mediator and inflammatory cytokine[9]. Some scholars have found that HMGB1 can be maintained and prolonged in the pathogenesis of sepsis and systemic inflammatory response and can be used as a key signal of cell selection for apoptosis and necrosis[10]. In animal experiments, other scholars have found that HMGB1 is significantly overexpressed in the bronchial epithelium of rats with COPD, which is consistent with the pathological changes of vascular structures in the lungs[11].

NE is mainly derived from neutrophils and exists in a small amount in monocytes and T cells. It is one of the most destructive enzymes and participates in the killing of Gram-negative bacilli under physiological conditions. During inflammation, NE released by activation of inflammatory cells can reduce coagulation factors and immunoglobulin, etc.[12] NE can induce epithelial cells to release a variety of pro-inflammatory cytokines such as IL-6 and IL-8, which can cause the decomposition of the kinin-releasing enzyme, fibrinogen, H factor and so on to form the activation product. NE can also induce differentiation and metaplasia of mucus cells, and a large amount of mucus secretion can lead to mucociliary clearance dysfunction and local defense function impairment[13].

IL-1β is mainly produced by mononuclear macrophages and endothelial cells, which can activate the inflammatory mediators released by macrophages and other inflammatory cells and promote the proliferation, activation, and differentiation of thymocytes. In addition, IL-1β also plays a regulatory role in the occurrence and development of COPD by promoting the secretion of IL-2 inflammatory cytokines by T cells[14-15].

The results of this study showed that the levels of HMGB1, NE, and IL-1β in the acute exacerbation group were significantly higher than those in the stable group and normal control group (P<0.05). The serum levels of HMGB1, NE, and IL-1β in the stable group were significantly higher than those in the normal control group (P<0.05), indicating that the more severe the disease, the higher the serum levels of HMGB1, NE, and IL-1β. The results of Pearson analysis showed that serum HMGB1, NE, and IL-1β were negatively correlated with pulmonary function indexes in patients with acute exacerbation and patients in the stable phase (P<0.05). The related reasons may be that the immune function of COPD

patients is disturbed to varying degrees, which further increases the inflammatory response of the body, leads to the increase of mucus secretions in the respiratory system, and affects the pulmonary ventilation function of patients.

In conclusion, the expression levels of HMGB1, NE, and IL-1β in the serum of COPD patients are all significantly increased and are significantly negatively correlated with FEV1/FVC and FEV1% PRED. These three indicators can reflect the severity of COPD patients to a certain extent and are worthy of clinical promotion.

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