

Research Article

Study on Regulation of Th17, Treg, Th17/Treg and Lung Function in Stable COPD Patients Treated with Acupoint Application

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- Acupoint Application
- Chronic Obstructive Pulmonary Disease
- T Helper Cell 17
- T Regular Cell
- Lung Function

Abstract

Objective: To investigate the effect of acupoint application in treating stable Chronic Obstructive Pulmonary Disease (COPD) patients and the effect on Th17, Treg and Th17/Treg expression and the improvement of lung function in.

Methods: A total of 100 patients with stable COPD admitted to Qiaotou Town Health Center, Datong County, Xining City, Qinghai Province from January to June in 2024 who were 1:1 randomly divided into experimental group and control group. The control group received conventional treatment, and the observation group received conventional treatment combined with acupoint application. Both groups received continuous treatment for 4 weeks. Th17, Treg, Th17/Treg expression, lung function, and CAT scores were compared between the two groups.

Results: (1) After treatment, Th17, Th17/Treg were lower than before treatment ($P < 0.001$), and Treg were higher than before treatment ($P < 0.05$). After treatment, Th17, Th17/Treg and Treg in the experimental group were better than those in the control group ($P < 0.05$). (2) The levels of IL-17 after treatment were lower than before treatment ($P < 0.05$), and the levels of TGF- β were higher than before treatment ($P < 0.05$). After treatment, the expression in the experimental group was better than that of the control group ($P < 0.05$). (3) Pulmonary function FEV1 and FEV1/FVC were significantly improved in the two groups after treatment ($P < 0.001$), and the improvement was more significant in the experimental group ($P < 0.05$). (4) After treatment, the CAT evaluation scores in both groups were decreased ($P < 0.05$), and the treatment group was more significant ($P < 0.05$).

Conclusion: Acupoint application combined with routine treatment in stable COPD patients has better efficacy, and can improve cellular immune function and lung function.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disease, which is characterized by irreversible airflow limitation, seriously affecting the activity and quality of life of patients. It is reported that COPD has become the third leading cause of death in the world, with a global prevalence of approximately 11.7% among people over 30 years old [1]. With the incidence rate sharply increasing with age, China is gradually moving towards aging and COPD is bound to increase the economic and social burden. How to better prevent and manage COPD, reduce its incidence and mortality rate, is a major task in clinical and basic research. Chronic inflammatory response

is one of the important mechanisms in the pathogenesis of COPD, in which the immune mechanism mediated by CD4⁺ T cells plays an important role. Both T helper cell 17 (Th17) and T regular cell (Treg) are differentiated from immature CD4⁺ T cells. Th17 can exacerbate COPD by secreting pro-inflammatory cytokines such as interleukin (IL)-17 and IL-21, while Treg can suppress the activation and release of immune cells by secreting anti-inflammatory cytokines such as transforming growth factor beta (TGF- β) and IL-10, thereby exacerbating the inflammatory response in the body. Acupoint application is to apply the paste of Chinese medicine to specific acupoints, and obtain certain curative effect in the stable period of COPD [2]. The aim of this study is to investigate the expression of Th17, Treg, Th17/Treg,

and related inflammatory factors in the treatment of stable COPD with acupoint application treatment.

MATERIALS AND METHODS

Materials

Objective: 100 COPD patients who visited the General Practice Department of Qiaotou Town Health Center, Datong County, Xining City, Qinghai Province from January to June in 2024 were selected as the research subjects. Patients were randomly divided into experimental group and control group at a 1:1 ratio according to a random number table, with 50 cases in each group. This study was approved by the Ethics Committee of Qiaotou Town Health Center in Datong County, Xining City, Qinghai Province.

Inclusion criteria: (1) COPD meets the revised diagnostic criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) in 2023[3]; (2) Age between 18 and 80 years old; (3) Severity of illness: Select COPD patients in the stable phase of COPD with moderate to severe symptoms, low risk of acute exacerbation, and no obvious pulmonary arterial hypertension; (4) Voluntarily accept this study and sign an informed consent form.

Exclusion criteria: (1) Patients with COPD developing into pulmonary encephalopathy stage. (2) Patients with airflow limitation due to bronchiectasis, cystic pulmonary fibrosis, active tuberculosis, and lung cancer. (3) Patients with severe fatal diseases such as acute heart failure, acute cerebral hemorrhage, gastrointestinal bleeding, aplastic anemia, acute renal failure, etc. (4) Pregnant or lactating women. (5) Patients with mental illness, severe neurological deficits such as aphasia, loss of recognition, or inability to cooperate due to other reasons. (6) For individuals who are allergic to research drugs.

Methods

Research methods: The control group received routine treatment, mainly including oxygen therapy, beta 2 receptor agonists, and anticholinergic drugs (7.2 ug of clotrimazole bromide and 5.0ug of formoterol, twice daily, twice a time, Astra Zeneca AB). The experimental group was treated with traditional Chinese medicine acupoint application based on the control group. The prescription for traditional Chinese medicine includes 10g of ephedra, 5g of schisandra, 5g of ginseng, 15g of astragalus, 15g of icariin, 15g of cornus, 5g of asarum, 30g of mustard seed, and 10g of sweet spring. Grind the above medication into a powder, sieve through an 80- mesh sieve, add ginger juice to make a paste, and make an acupoint plaster. Take Feishu, Pishu, Shenshu, Fengmen, Danzhong, and Tiantu as the acupoints for application, once a day for 8 hours each time. Both groups of patients were treated continuously for 4 weeks.

Outcome measures

- (1) **Th17 and Treg cell detection:** The research subjects were all drawn 5 ml of vein blood on an empty stomach for more than 8 hours in the morning, anticoagulated with heparin, and peripheral blood mononuclear cells (PBMCs) were extracted using density gradient centrifugation. Two PBMCs were collected and added with anti-CD4 and anti-CD25 antibodies, respectively. They were incubated at 4°C in the dark for 30 minutes, washed, fixed, and punctured, and then added with anti-Foxp3, IL-17, and isotype control antibodies, respectively. They were incubated at room temperature in the dark for 60 minutes. After rinsing, Th17 and Treg were detected using a BD FACS Calibur flow cytometer, then the Th17/Treg ratio was calculated.
- (2) **IL-17 and TGF- β cytokine detection:** The blood collection method is the same as above. 200 ul of the blood supernatant were collected and centrifuged at 20°C and a speed of 200/min for 4 minutes. The concentration of cytokines in serum were detected by ELISA method (Beijing Baiaolaibo Technology Co., Ltd.).
- (3) **Pulmonary function test:** The test was completed by the General Medical Examination Room of our hospital, equipped with a pulmonary function tester (X2 from Kexiamen Medical Equipment Co., Ltd.). Pulmonary function indicators include forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and calculated first time lung capacity/forced vital capacity (FEV1/FVC).
- (4) **CAT score:** The patient's symptoms were evaluated based on six subjective aspects including cough, sputum production, sleep, chest tightness, emotions, and energy, as well as the impact of daily exercise and exercise endurance. The total score was 40 points. Among them, 0-10 points are mild conditions, 11-20 points are moderate conditions, 21-30 points are severe conditions, and 31-40 points are very severe conditions, unable to engage in any activities and completely unable to take care of oneself.

Statistical analysis

Data were analyzed using SPSS 24.0 statistical software. The measurement data was expressed as mean \pm standard deviation, and t-test was used for inter group comparison; Count data was presented in terms of examples and percentages (n [%]), and comparison between groups was

performed using χ^2 test. The difference was statistically significant with $P < 0.05$.

RESULTS

General Information Comparison

There was no statistically significant difference in gender, age, disease duration, body mass index, and smoking index between the two groups ($P > 0.05$), comparable (Table 1).

Comparison of Th17, Treg, Th17/Treg Before and after Treatment between Two Groups

Th17 and Th17/Treg levels in both groups of patients were significantly lower than before treatment ($P < 0.001$), and Treg levels were higher than before treatment ($P < 0.05$); The Th17 and Th17/Treg levels in the experimental group were significantly lower than before the control group ($P < 0.001$), and Treg levels were higher than those in the experimental group ($P < 0.05$) (Table 2).

IL-17 and TGF- β Cytokines Before and after Treatment

The levels of IL-17 in both groups of patients were lower than before treatment ($P < 0.05$), and the average level of TGF- β was higher than before treatment ($P < 0.05$); The IL-17 level in the experimental group was lower than that in the control group ($P < 0.05$), and the TGF- β level was higher than that in the control group ($P < 0.05$) (Table 3).

FEV1/FVC and FEV1/FVC before and after Treatment

The experimental group showed significant improvement in FEV1, FVC, and FEV1/FVC levels after treatment compared to before treatment ($P < 0.001$), while the control group showed significant improvement in FEV1 and FEV1/FVC levels after treatment compared to before treatment ($P < 0.001$); After treatment, the levels of FEV1 and FEV1/FVC in the experimental group improved compared to the control group ($P < 0.05$) (Table 4).

Table 1: General information comparison between the two groups.

	Male (%)	Age (Year)	Disease Duration (Year)	Body Mass Index (Kg/ M ²)	Smoking Index
Experimental group (N = 50)	29(58%)	61.32 ± 11.11	3.99 ± 2.54	22.01 ± 4.93	254.16 ± 295.41
Control group (N = 50)	23(46%)	60.50 ± 13.78	4.41 ± 2.91	21.40 ± 4.44	224.36 ± 272.54
F/χ^2 值	1.442	0.328	-0.769	0.863	0.524
P 值	0.230	0.744	0.444	0.390	0.601

Table 2: Comparison of Th17, Treg, and Th17/Treg levels in peripheral blood of two groups before and after treatment ($x \pm s$).

Experimental Group (N = 50)			Control Group(N = 50)		t	P
Th17	Before	4.07 ± 0.63	Before	4.08 ± 0.63	-0.113	0.910
	After	2.21 ± 0.54	After	2.75 ± 0.44	-5.540	0.000
	t	15.510	t	12.336	-	-
	P	0.000	P	0.000	-	-
Treg	Before	1.98 ± 0.62	Before	2.06 ± 0.66	-1.576	0.121
	After	2.40 ± 0.70	After	2.11 ± 0.66	2.404	0.020
	t	-3.326	t	-13.545	-	-
	P	0.002	P	0.000	-	-
Th17/Treg	Before	2.37 ± 1.10	Before	2.26 ± 1.03	1.390	0.171
	After	1.02 ± 0.41	After	1.46 ± 0.58	-4.447	0.000
	t	8.207	t	8.950	-	-
	P	0.000	P	0.000	-	-

Table 3: Comparison of IL-17 and TGF- β cytokines in peripheral blood of two groups of patients before and after treatment ($x \pm s$).

Experimental group (N = 50)			Control group (N = 50)		<i>t</i>	<i>P</i>
IL-17	Before	24.55 ± 3.84	Before	24.65 ± 4.00	-0.265	0.792
	After	23.50 ± 4.12	After	24.64 ± 4.00	-2.346	0.023
	<i>t</i>	5.304	<i>t</i>	3.055	-	-
	<i>P</i>	0.000	<i>P</i>	0.004	-	-
TGF-β	Before	34.01 ± 3.23	Before	33.70 ± 3.60	0.495	0.623
	After	34.80 ± 3.34	After	33.70 ± 3.60	2.085	0.042
	<i>t</i>	-2.198	<i>t</i>	-2.064	-	-
	<i>P</i>	0.033	<i>P</i>	0.044	-	-

Table 4: Changes in lung function before and after treatment in two groups of patients ($x \pm s$).

Experimental group(N= 50)			Control group(N= 50)		<i>t</i>	<i>P</i>
FEV1 (V/L)	Before	1.36 ± 0.25	Before	1.68 ± 0.31	-1.033	0.307
	After	2.43 ± 0.50	After	1.77 ± 2.22	2.027	0.048
	<i>t</i>	-14.115	<i>t</i>	-3.934	-	-
	<i>P</i>	0.000	<i>P</i>	0.000	-	-
FVC (V/L)	Before	2.30 ± 0.41	Before	2.77 ± 3.71	-0.924	0.360
	After	3.68 ± 0.77	After	2.80 ± 3.71	1.643	0.107
	<i>t</i>	-12.385	<i>t</i>	-0.814	-	-
	<i>P</i>	0.000	<i>P</i>	0.420	-	-
FEV1/FVC (%)	Before	59.28 ± 4.53	Before	60.61 ± 3.22	-1.876	0.067
	After	66.17 ± 6.0	After	63.97 ± 3.65	2.339	0.023
	<i>t</i>	-9.928	<i>t</i>	-16.039	-	-
	<i>P</i>	0.000	<i>P</i>	0.000	-	-

Table 5: Comparison of CAT scores between two groups of patients before and after treatment ($x \pm s$, s/score).

	Experimental Group (N = 50)	Control Group (N = 50)	t	P
Before	20.54 ± 6.25	20.42 ± 6.33	0.195	0.846
After	18.82 ± 5.59	20.20 ± 6.48	-2.112	0.04
t	8.114	3.07	-	-
P	0	0.003	-	-

Comparison of CAT Scores between Two Groups of Patients before and after Treatment

Both groups of patients showed a decrease in CAT scores after treatment compared to before treatment ($P < 0.05$). After treatment, the scores of the experimental

group patients further decreased compared to the control group ($P < 0.05$) (Table 5).

CONCLUSIONS

COPD is a common chronic respiratory disease in clinic, which is included in the category of “lung distension” in Traditional Chinese Medicine (TCM). In TCM, “qi” is a fundamental concept that refers to the vital energy flowing through the body. It is considered that after a long period of lung function deficiency, the main “qi” is not favorable, the lung “qi” is stagnant, and children’s illness harms the mother, affecting the spleen and kidneys. Therefore, lung and kidney deficiency are the fundamental causes of COPD, and phlegm turbidity is the hallmark of the disease. Acupoint application is guided by the theory of tonifying the lung and kidney, promoting lung function, and relieving cough in TCM. Based on the meridian theory and holistic view, TCM is applied to corresponding acupoints to stimulate them, inhale drugs through the skin, and regulate yin and yang through meridian conduction for treatment. Feishu acupoint replenishes lung qi; Spleen Yu acupoint can invigorate qi and promote clarity; Shen Shu acupoint warms Yang and absorbs Qi; Fengmen acupoint is the gateway for wind evil to enter and exit, nourishing qi and stabilizing the surface; The Danzhong acupoint is a place where the Qi of the sect gathers and regulates the Qi machine; The Tian Tu acupoint can widen the chest, regulate qi, lower qi, and resolve phlegm [4,5].

The mechanism of the TCM applied in this experiment is to warm up phlegm, regulate qi and relieve asthma, and use heat and yang warming drugs to assist the body’s yang energy, in order to resist cold pathogens in autumn and winter and reduce the incidence of diseases. Yin Yaqin, et al. included 42 Randomized Controlled Trials (RCTs) with a total of 4192 patients. Meta analysis showed that adding acupoint application therapy to conventional treatment significantly improved the effective rate [RR = 1.23, 95% CI (1.19, 1.27), $P < 0.001$] and increased FEV1/FVC [MD = 4.46, 95% CI (3.17, 5.76), $P < 0.001$]. The improvement of FEV1 and FEV1/FVC was significantly higher in the experimental group than in the control group, and the active evaluation of the experimental group was also significantly higher than that of the control group after treatment, with statistically significant differences ($P < 0.05$), indicating that acupoint application is effective for stable COPD and can significantly improve patients’ lung function and daily living ability.

Th17 and Treg are key cells for immune regulation in the body, and a significant imbalance of Th17/Treg can accelerate the occurrence and development of COPD.

The number of Th17 cells in COPD patients is relatively increased, releasing a large amount of pro-inflammatory cytokines; the relative decrease in Treg count and secretion of anti-inflammatory factors accelerate the progression of the disease, and this change dynamically changes with the severity of the disease. Therefore, the dynamic changes between Th17 and Treg have certain guiding significance for grasping disease activity and treatment [6,7]. Xu, et al., confirmed that ginsenosides can improve the inflammatory response of COPD by upregulating FOXP3, increasing Treg expression, and reducing Th17 expression from animal and human experiments. Smoking-induced COPD mice showed increased expression of pro-inflammatory factors (D4, CD8, CD20, IL-17, etc.) and decreased anti-inflammatory immune responses (FOXP3, TGF- β , etc.) compared to the control group, suggesting that Th17/Treg imbalance promotes COPD progression [8,9].

Secreted frizzled related protein 2 (sFRP2) is significantly upregulated in the serum and bronchial epithelial cells of COPD patients, increasing Th17% in CD3+CD8- cells and decreasing Treg% in CD4+CD25+ cells. sFRP2 enhances airway inflammation, disrupts Th17/Treg homeostasis, and promotes the progression of COPD [10]. Based on the above research, we aim to explore the immune mechanism of TCM acupoint application by examining Th17/Treg balance and cytokine secretion. The results suggest that: (1) After 4 weeks of acupoint application therapy in stable COPD patients, the Th17/Treg balance was improved, and it was better than the simple drug treatment group. (2) After acupoint application therapy, the expression of pro-inflammatory cytokine IL-17 was significantly downregulated, while the expression of anti-inflammatory cytokine TGF- β increased, thereby improving the chronic inflammatory state of stable COPD patients. (3) Due to the fact that acupoint application therapy has improved the immune function of stable COPD patients to a certain extent, thereby promoting the recovery of lung function, patients’ active evaluation of cardiovascular and pulmonary function in daily life is better.

This study has certain limitations: (1) due to time constraints, the sample size included in the study was not sufficient, and no grouping analysis was conducted on the improvement of COPD in different age groups; (2) Only moderate to severe COPD patients were included, and mild patients were not included, which may be related to the low visit rate of mild patients; (3) Some middle-aged and elderly COPD patients inevitably suffer from other underlying diseases, which may have a certain impact on the research results. Therefore, further expansion of the sample size is needed for in-depth research.

In summary, the balance of Th17/Treg plays an important role in the development of chronic inflammation in COPD patients. Under standard standardized treatment, combined with traditional Chinese medicine acupoint application therapy, it may become an effective treatment method to improve the imbalanced immune state, play the role of traditional Chinese medicine in COPD treatment, reduce drug side effects, and reduce acute attacks in stable COPD patients.

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