Effect of lower airway bacterial colonization on immune function, airway function and degree of inflammation in patients with stable COPD

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1. Introduction

Before suffering from pathogen infection and acute onset of airway inflammation, patients with chronic obstructive pulmonary disease (COPD) can mostly be in stable stage, and maintaining patients with COPD in stable stage for a long time is the key to avoid the ultimate respiratory failure[1]. Human respiratory tract is sterile under physiological conditions, and the related research has found that the number of flora colonized in lower respiratory tract of patients with stable COPD is positively correlated with the number of cigarettes smoked and the concentration of inflammatory factors. After colonization, the pathogenic bacteria can further cause declined cilia movement ability, reduced macrophage phagocytosis, increased mucus secretion and decreased airway purification ability in patients with COPD, and therefore, some scholars put forward whether the lower airway bacterial colonization is one of the key factors deciding the ultimate treatment outcome of patients.
with COPD[2,3]. In order to define the specific effect of the lower respiratory tract flora colonization on the patients with COPD, the number of pathogenic bacteria in lower respiratory tract of patients with stable COPT and healthy subjects was tested in the study, and the differences in immune function, airway function, the degree of inflammation and so on were further analyzed between different groups, hereby reported as follows.

2. Materials and methods

2.1. General information

A total of 60 patients with stable COPD treated in our hospital between February 2014 and February 2016 were selected as observation group, and the inclusion criteria were: (1) in accordance with the WHO-established diagnostic criteria for COPD; (2) without medical history of acute onset within last month; (3) stopped using glucocorticoids, antibiotics and theophylline drugs within 72 h before research; (3) stopped using β receptor agonist and anticholinergic inhibitors within 12 h before research; (4) the patients signed informed consent for research process; (5) completed the research process and didn’t drop out. Exclusion criteria: (1) with severe respiratory failure; (2) with severe liver and kidney function damage; (3) associated with autoimmune diseases; (4) associated with systemic serious infectious diseases. 60 healthy subjects who received physical examination in our hospital and were with normal lung function were selected as control group. Observation group included 35 male cases and 25 female cases that were 46-72 years old and (60.28±8.95) years old in average; control group included 32 male cases and 28 female cases that were 44-71 years old and (58.93±7.65) years old in average. The two groups of subjects showed no statistically significant difference in the distribution of gender and age (P>0.05).

2.2. Low respiratory tract–colonized bacterial culture

Subjects rinsed mouth and then deeply coughed, and the sputum of first cough was collected, put in a sterile bottle, inspected and observed under the low-power field. Sputum pretreatment liquid and qualified sputum (number of squamous cells <10 and number of polynuclear leucocyte > 25) were mixed in 1:1 ratio and prepared into stock solution. The stock solution was doubling-diluted, made into diluent, put in Petri dish and inoculated to culture bacteria. Bacteria number ≥10^7 CFU/mL indicated the existence of lower airway bacterial colonization.

2.3. Observation indexes

2.3.1. Peripheral blood indexes

A total of 3 mL of fasting cubital venous blood was collected from all the research subjects in the morning. 1ml was used to determine white blood cell CD34^+ , CD64 and CD11b content as well as helper T cell (Th17) and regulatory T cells (Treg) content by flow cytometer. The remaining 2 mL of peripheral blood was let stand at room temperature for 20-30 min and centrifuged at 3 500 r/min for 5-10 min, supernatant was collected and then cryopreserved in -70°C refrigerator for test, and the specific detection indexes were as follows: (1) inflammatory factors: ELISA method was used to determine C-reactive protein (CRP), interleukin-1β (IL-1β), interleukin-8 (IL-8) and interleukin-17 (IL-17) and interleukin-27 (IL-27) content; (2) airway remodeling indexes: ELISA method was used to determine vascular endothelial growth factor (VEGF), transforming growth factor 1 (TGF-1), nerve growth factor (NGF), basic fibroblast growth factor (b-FGF), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) content.

2.3.2. Small airway function indexes

Swiss Schiller instrument Spirovit SP-1M was used to determine the small airway function of both groups, including forced expiratory flow at 25% of forced vital capacity (FEF25), forced expiratory flow at 50% of forced vital capacity (FEF 50), forced expiratory flow at 75% of forced vital capacity (FEF 75) and forced expiratory flow at 25%-75% of forced vital capacity (FEF25-75).

2.4. Statistical methods

Data in the study was input in software SPSS23.0, measurement data analysis among three groups was performed by variance analysis and pair-wise comparison was by LSD–t test, and P<0.05 indicated statistical significant differences.

3. Results

3.1. Bacterial culture results

Except normal oral flora, 60 cases of control group were without pathogenic bacteria. 35 cases in observation group were with lower airway bacterial colonization, and the main flora included Streptococcus pneumoniae (2.8 10^7/CFU/mL), Pseudomonas aeruginosa (1.9 10^7/CFU/mL), Staphylococcus aureus (2.9 10^7/CFU/mL), Haemophilus influenzae (1.3 10^7/CFU/mL) and Haemophilus parainfluenzae (3.1 10^7/CFU/mL). The remaining 25 cases had no lower airway bacterial colonization.
3.2. Immune function indexes

CD34+ and CD11b content in peripheral blood of non-colonization group were significantly lower than those of control group while CD64, Th17 and Treg content were significantly higher than those of control group (P<0.05). CD34+ and CD11b content in peripheral blood of colonization group were significantly lower than those of non-colonization group and control group (P<0.05) while CD64, Th17 and Treg content were significantly higher than those of non-colonization group and control group (P<0.05), shown in Table 1.

3.3. Small airway function

FEF25, FEF50, FEF75 and FEF25-75 levels of non-colonization group were significantly higher than those of non-colonization group and control group while FEF25, FEF50, FEF75 and FEF25-75 levels of colonization group were significantly lower than those of control group (P<0.05), shown in Table 2.

3.4. Inflammatory factor content

CRP, IL-1β, IL-8, IL-17 and IL-27 content in serum of non-colonization group were significantly higher than those of non-colonization group and control group (P<0.05); CRP, IL-1β, IL-8, IL-17 and IL-27 content in serum of colonization group were significantly higher than those of non-colonization group and control group (P<0.05), shown in Table 3.

3.5. Airway remodeling indexes

VEGF, TGF-1, NGF, b-FGF, MMP-9 and TIMP-1 content in serum of non-colonization group were significantly higher than those of control group (P<0.05); VEGF, TGF-1, NGF, b-FGF, MMP-9 and TIMP-1 content in serum of colonization group were significantly higher than those of non-colonization group and control group (P<0.05), shown in Table 4.

4. Discussion

Pathogenic bacterial colonization in lower respiratory tract is one of the important factors for progression of stable COPD and acute onset of respiratory tract infections, and long-term smokers have higher probability of pathogenic bacterial colonization in lower respiratory tract, which may be related to airway epithelial damage.
increased micro-environmental toxin release and enhance bacteria and epithelial cell adhesion[4]. Related studies have shown that the top three pathogens colonized in respiratory tract of patients with COPD are Haemophilus parainfluenzae, Streptococcus pneumoniae and Branhamella catarrhalis, the types and number of pathogenic bacteria in lower respiratory tract of patients with stable COPD in our hospital were also detected in the study, it was found that 35 cases out of 60 cases were with pathogenic bacteria colonization, and the colonized flora with the largest number were Haemophilus parainfluenzae, Staphylococcus aureus and Streptococcus pneumoniae, which is slightly different from the previous literature report and may be associated with the differences in specific living and hospitalization environment. In order to define the impact of lower respiratory tract pathogenic bacterial colonization on patients with COPD, immune function, airway function, degree of inflammation, degree of airway remodeling, and so on and so forth of colonization group, non-colonization group and control group were detected respectively in the study.

Declined immune function and decreased immune cell attack on pathogenic bacteria are the important reasons for protraction and even worsening of COPD. Many scholars put forward that in addition to the external environment pollution, the declined self-purification ability of the body as well as white blood cell, mononuclear macrophage and other innate immune barrier dysfunction are the key precipitating factors of the pathogenic bacteria colonization in lower respiratory tract of patients with COPD[5,6]. CD34 cells are involved in the construction of the immune function of patients with COPD, and study has found that CD34 cell content declines sharply in patients with severe COPD. CD64 belongs to the immunoglobulin superfamily, can specifically recognize immunoglobulin fragments, and is highly expressed when the body is in a stress state[7]. CD11b content is consistent with that of macrophages, and CD11b is also lowly expressed in the case of macrophage function impairment. Th17 can secrete IL-17 to exert pro-inflammatory effect, and Th17 is also confirmed to play an important role in the immune response of in emphysema caused by smoking[8]. Tregs is the T cell subgroup with suppressive function, and can inhibit effector T cell proliferation and activation and reduce the production of B cell immunoglobulin. In the study, detection of the content of above immune indexes showed that CD34 content and CD11b content in peripheral blood of colonization group were the lowest while the CD64, Th17 and Treg content were the highest, confirming that after there is pathogenic bacteria colonization in lower respiratory tract of patients with stable COPD, body’s immune response ability significantly reduces, and it is the important reason for the later disease deterioration.

COPD mainly involves small airway, so the detection of small airway function can be used as the gold standard for COPD judgment. FEEF25, FEF50, FEF75 and FEF25-75 are the most common indicators for clinical judgment of small airway function status[9,10]. In the study, the levels of above indexes of the three groups were tested, and it was found that FEF25, FEF50, FEF75 and FEF25-75 levels of patients with COPD were lower, and the FFEF25, FEF50, FEF75 and FEF25-75 levels of colonization group were the lowest, indicating that patients with COPD are with small airway function damage, and when the patients with COPD are with concurrent pathogenic bacteria colonization in lower respiratory tract, patients’ small airway anatomy and function injury continue to increase, which is the visual performance of ventilation and air exchange dysfunction in patients. Local airway and systemic inflammation are the important precipitating reasons for disease progression and respiratory function decline in patients with stable COPD. When there is pathogenic bacteria colonization in lower respiratory tract, the colonized pathogenic bacteria can cause chronic airway inflammation, increase local airway secretions and further increase the number of colonized bacteria, and the three form a vicious circle[11,12]. In the study, the serum content of inflammatory factors of three groups of research subjects were tested, and it was found that pro-inflammatory factors such as CRP, IL-1β, IL-8, IL-17 and IL-27 content in serum were higher in patients with COPD, and CRP, IL-1β, IL-8, IL-17 and IL-27 content in serum further increase in patients with pathogenic bacteria colonization in lower respiratory tract, indicating that there is severe local inflammation in such patients, and it is the aura of COPD progression.

Increased bronchial reticular basement membrane thickness is one of the characteristic changes of COPD, and along with the persistent increase of basement membrane thickness, patients’ respiratory function progressively decreases. There is different degree of airway remodeling in patients with COPD, it is the pathological basis of irreversible airway limitation in such patients, and VEGF, TGF-1, NGF, b-FGF, MMP-9 and TIMP-1 are even directly involved in the occurrence and development of airway remodeling[13,14]. VEGF can lead to endothelial cell proliferation, angiogenesis and increased permeability. Studies have found that the mononuclear macrophages in airway mucosa of patients with COPD can massively secrete NGF and participate in regulation of neutrophils. b-FGF can regulate the proliferation of neutrophils, mastocytes, macrophages and so on, and is directly involved in the airway remodeling process[15,16]. MMP-9 and TIMP-1 are involved in the degradation and reconstruction of extracellular matrix, and TIMP-1, as inhibitor of MMP-9, can cause extracellular matrix deposition, lead to the accumulation of collagen, fibronectin and others in the submucosa, and cause airway thickening[17]. It was found in the study that VEGF, TGF-1, NGF, b-FGF, MMP-9 and TIMP-1 content in serum of colonization group were significantly higher than those of not-colonization group and control group, indicating that the pathogenic bacteria colonization in lower respiratory tract can lead to more serious airway remodeling. 
in patients with COPD and set the stage for long-term acute COPD onset, respiratory failure and so on.

To sum up, lower airway bacterial colonization in patients with stable COPD can cause immune suppression as well as airway inflammation and remodeling, and accelerate disease progression.

References


