Original Article

Antimicrobial peptide LL-37 circulating levels in chronic obstructive pulmonary disease patients with high risk of frequent exacerbations

Yi-Meng Yang, Yan-Fei Guo, Hong-Sheng Zhang, Tie-Ying Sun

Department of Respiratory and Critical Care Medicine, Beijing Hospital, Beijing 100730, China

Correspondence to: Tie-Ying Sun. Department of Respiratory and Critical Care Medicine, Beijing Hospital, Beijing 100730, China. Email: suntieying@126.com.

Background: Exacerbations of chronic obstructive pulmonary disease (COPD) increase the decline in lung function, deterioration in health status and risk of death. The assessment of exacerbation risk is important in the grading of COPD. The most common cause of COPD exacerbation is respiratory tract infection. The only known human cathelicidin antimicrobial peptide, LL-37, play an important role in innate defense against infection. Its gene expression is regulated by the bioactive form of vitamin D. The objective of the present study was to explore the relationship between LL-37 plasma levels, vitamin D status and exacerbation risk in patients with COPD.

Methods: COPD patients and normal subjects were recruited from Beijing Hospital for this study. COPD patients were divided into low risk group and high risk group according to the criteria of GOLD strategy. The plasma concentrations of LL-37 were measured by ELISA technique to explore the difference in LL-37 levels between groups. The plasma levels of 25-hydroxy vitamin D [25(OH)D] were analyzed using electrochemiluminescence immunoassay (ECLIA).

Results: A total of 84 COPD patients and 51 normal subjects (control group) were recruited. COPD patients were divided into low risk group (37 cases) and high risk group (47 cases), depending on forced expiratory volume in one second (FEV_1)%pred and exacerbation frequency in the previous year. The plasma concentrations of LL-37 in control group, low risk group and high risk group were 20.7±5.8, 19.5±4.1 and 17.9±3.9 μg/L respectively. The plasma concentration of LL-37 was significantly lower in high risk group than in control group (P=0.006). But there was no significant difference between low risk group and high risk group (P=0.152). The plasma concentrations of 25(OH)D in control group, low risk group and high risk group were 18.1±9.4, 13.1±6.9 and 9.3±5.8 ng/mL respectively. The plasma concentration of 25(OH)D was significantly higher in control group than in low risk group (P=0.004) or high risk group (P<0.001). The plasma concentration of 25(OH)D was significantly lower in high risk group than in low risk group (P=0.031). Hospitalization frequency for COPD exacerbations was negative correlated with plasma levels of LL-37 (r=−0.290, P=0.048) and 25(OH)D (r=−0.341, P=0.020) in high risk group. There was not significant correlation between LL-37 and 25(OH)D in COPD patients (r=0.115, P=0.303).

Conclusions: The plasma levels of LL-37 and 25(OH)D were lower in COPD patients with high risk of frequent exacerbations than normal subjects. Low plasma levels of LL-37 and 25(OH)D might be predictors of exacerbation risk in COPD patients.

Keywords: Chronic obstructive pulmonary disease (COPD); exacerbation risk; antimicrobial peptide; LL-37; vitamin D

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Introduction

Chronic obstructive pulmonary disease (COPD), the fourth leading cause of death in the world, represents an important public health challenge that is both preventable and treatable (1). Exacerbations of COPD increase the decline in lung function, deterioration in health status and risk of death (2-6). The new Global Initiative for Chronic Obstructive Lung Disease strategy (GOLD strategy 2013) included the number of exacerbations, especially severe exacerbations requiring hospital admission, in the grading of COPD (1). The most common cause of COPD exacerbation appears to be respiratory tract infection (viral or bacterial). Host defense peptides are ancient weapons of the innate immunity. In mammals, antimicrobial peptides of the defensin and the cathelicidin families are found. These peptides are expressed on epithelial surfaces and in neutrophils and have been proposed to provide a first line of defence against infection. The only human cathelicidin, human cationic antimicrobial protein 18 (hCAP18), and its C-terminal 37 amino acid fragment (LL-37) have multiple functions, including microbial killing, neutralizing lipopolysaccharide, stimulating leukocyte chemotaxis, promoting angiogenesis and wound healing (7-9). Some studies found that airway concentration of LL-37 (sputum, bronchoalveolar lavage fluid) is up regulated in COPD patients (10,11). However, the relationship between circulating levels of LL-37 and exacerbation risk in COPD patients have not received much attention. The 25-hydroxy vitamin D [25(OH)D] deficiency is associated with COPD and increased susceptibility to infection in the general population (12). In addition, the human cathelicidin antimicrobial peptide gene expression is regulated by the bioactive form of vitamin D (13,14). The objective of our study was to explore the relationship between LL-37 plasma levels, vitamin D status and exacerbation risk in COPD patients.

Methods

Subjects

COPD patients and healthy subjects were recruited from Beijing Hospital, Peking University, China. COPD patients were diagnosed according to the criteria of GOLD strategy (1). The presence of a post-bronchodilator forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) <0.70 confirms the presence of persistent airflow limitation. All patients were in stable clinical condition without reported exacerbation within three months. An exacerbation of COPD is defined as an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication (1). COPD patients were divided into low risk group (FEV_1%pred ≥50% and <2 treated exacerbation per year was considered as low risk) and high risk group (FEV_1%pred <50% or ≥2 treated exacerbations per year or ≥1 hospitalizations for COPD exacerbations per year were considered as high risk), depending on the criteria of GOLD strategy (1). Patients who had conditions known to affect plasma concentrations of LL-37 and 25(OH)D, such as cancer, collagen vascular disease, other infection disease, other respiratory disease except COPD were strictly excluded. Healthy subjects had normal physical examinations and showed no symptoms or signs of infection at the time of study. Spirometric test was evaluated in all participants. Data regarding the number of exacerbations in the previous year, smoking history and respiratory symptoms (Modified British Medical Research Council questionnaire, mMRC questionnaire) of COPD patients were evaluated. All participants gave written informed consent with protocols approved by the Institutional Review Boards of Beijing Hospital.

Pulmonary function tests

Pulmonary function tests were performed according to American Thoracic Society guidelines for performance (15). FEV_1 and FVC were measured before and after inhaled bronchodilator with standard spirometric techniques (Vmax62 Sensor Medics, Calif, USA). The highest value from at least three spirometric maneuvers was used.

Measurement of LL-37 and vitamin D

Peripheral venous blood samples were taken from all participants. Plasma was separated from blood cells by centrifugation at 1,500 g for 15 min. All samples were stored at −80 ℃ for subsequent analyzed. The plasma concentrations of LL-37 were measured using ELISA technique (Hycult Biotech, Uden, the Netherlands). The plasma levels of 25(OH)D were measured by electrochemiluminescence immunoassay (ECLIA) on a COBAS e601 ROCHE® analyzer.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) unless otherwise indicated. Statistical comparisons were made

Table 1 Patient characteristics

Characteristics	Control group	Low risk group	High risk group
Total No. subjects	51	37	47
Male/female (No.)	25/26	24/13	37/10
Mean age, y ($\bar{x}\pm s$)	68.8±7.3	68.6±8.2	69.9±9.3
Smoker/non-smoker (No.)	26/25	25/12	43/4
FVC (L) ($\bar{x}\pm s$)	2.98±0.69	3.01±0.88	2.33±0.67*,†
FVC%pred (%) ($\bar{x}\pm s$)	99.3±17.3	89.5±23.4*	68.4±15.3*,†
FEV_1 (L) ($\bar{x}\pm s$)	2.43±0.60	1.80±0.71*	1.15±0.50*,†
FEV_1%pred (%) ($\bar{x}\pm s$)	100.0±16.9	69.8±25.1*	44.7±18.2*,†
FEV_1/FVC (%) ($\bar{x}\pm s$)	82.1±4.9	57.7±11.3*	47.9±12.3*,†
mMRC score (median)	0	0	3
Exacerbations per year (median)	0	0	2
Hospitalizations for COPD exacerbations per year (median)	0	0	1

*, there is significant difference compare with control group, P<0.05; †, there is significant difference compare with low risk group, P<0.05. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; mMRC, Modified British Medical Research Council; COPD, chronic obstructive pulmonary disease.

using analysis of variance for multiple group comparisons. The relations between variables were evaluated with Pearson's correlation. A P value <0.05 was considered statistically significant. Data analyses and descriptive statistics were performed with the statistical package for social sciences (SPSS 13.0).

Results

Subject groups

A total of 84 COPD patients and 51 normal subjects (control group) were recruited. COPD patients were divided into low risk group (37 cases) and high risk group (47 cases), depending on FEV_1%pred and exacerbation frequency in the previous year. In high risk group, 93.6% patients have had once or more hospitalizations for COPD exacerbations within previous year. There was no significant difference of participants' age between groups (F=0.334, P=0.717). The post-bronchodilator FEV_1%pred and FEV_1/FVC were significantly higher in control group than in High Risk group or Low Risk group (P<0.001 for each). The post-bronchodilator FEV_1%pred and FEV_1/FVC were significantly lower in high risk group than in low risk group (P<0.001). Patient characteristics are shown in *Table 1*.

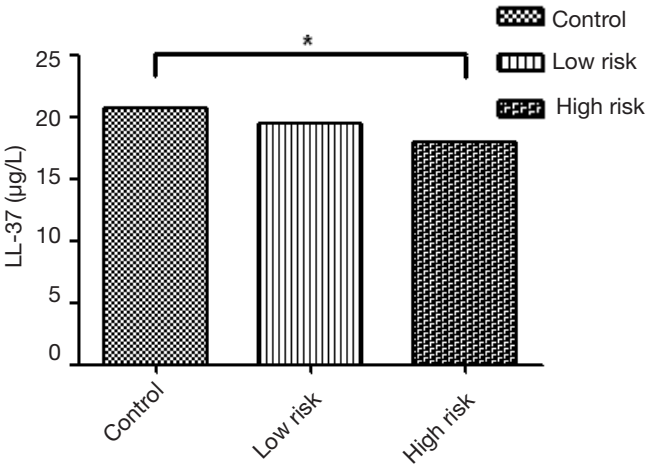

Figure 1 The plasma levels of LL-37 in control group, low risk group and high risk group. *, plasma levels of LL-37 were significantly lower in high risk group than in control group.

LL-37 levels in plasma

The plasma concentrations of LL-37 in control group, low risk group and high risk group were 20.7±5.8, 19.5±4.1 and 17.9±3.9 μg/L respectively (*Figure 1*). The plasma concentration of LL-37 was significantly lower in high risk group than in control group (mean difference −2.75, 95% CI, −4.69 to −0.82, P=0.006). But there was no significant difference between low risk group and high risk group (mean difference 1.53, 95% CI, −0.57 to 3.63, P=0.152). There was no significant difference between low risk group and control group in plasma concentration of LL-37 (mean difference −1.22, 95% CI, −3.27 to 0.82, P=0.239).

25-hydroxy vitamin D [25(OH)D] levels

The plasma concentrations of 25(OH)D in control group, low risk group and high risk group were 18.1±9.4, 13.1±6.9 and 9.3±5.8 ng/mL respectively (*Figure 2*). The plasma concentration of 25(OH)D was significantly higher in control group than in Low Risk group (mean difference 4.98, 95% CI, 1.66-8.30, P=0.004) or high risk group (mean difference 8.75, 95% CI, 5.61-11.89, P<0.001). The plasma concentration of 25(OH)D was significantly lower in high risk group than in low risk group (mean difference −3.78, 95% CI, −7.18 to −0.36, P=0.031).

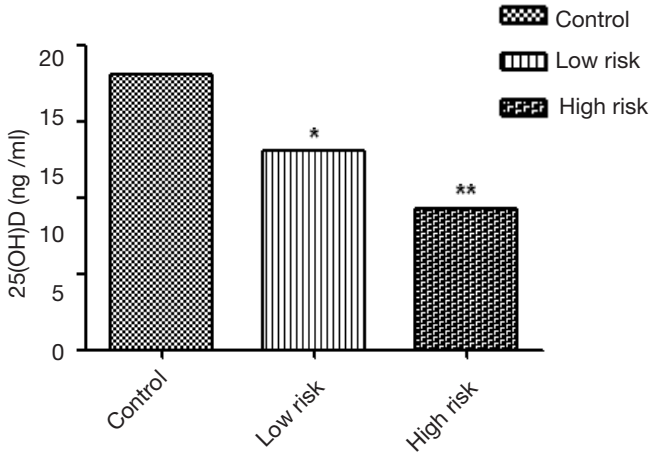

Figure 2 The plasma levels of 25(OH)D in control group, low risk group and high risk group. *, the plasma level of 25(OH)D was significantly lower in low risk group than in control group; **, the plasma level of 25(OH)D was significantly lower in high risk group than in low risk group or control group. 25(OH)D, 25-hydroxy vitamin D.

LL-37, 25(OH)D and exacerbation risk

Exacerbation frequency was negative correlated with FEV_1%pred (r=−0.395, P=0.001), FEV_1/FVC (r=−0.412, P<0.001) and 25(OH)D (r=−0.341, P=0.003) in COPD patients. In high risk group, hospitalization frequency for COPD exacerbations per year was negative correlated with plasma levels of LL-37 (r=−0.290, P=0.048) and 25(OH)D (r=−0.341, P=0.020). There was no significant correlation between LL−37 and 25(OH)D (r=0.115, P=0.303) in COPD patients. The relationship between LL-37, 25(OH)D, age, lung function, syndrome and exacerbation frequency in COPD patients are shown in *Tables 2* and *3*.

Table 2 The relationship between LL-37, 25(OH)D and exacerbation risk in COPD patients

Variables	LL-37		25(OH)D	
	r	P	r	P
25(OH)D	0.115	0.303	1	
Age	0.154	0.162	0.039	0.725
FEV_1%pred	0.132	0.247	0.343*	0.002
FEV_1/FVC	0.036	0.756	0.317*	0.005
Exacerbations per year	−0.227	0.052	−0.341*	0.003
mMRC score	−0.282	0.096	−0.277	0.112

*, there is correlation between two indexes. 25(OH)D, 25-hydroxy vitamin D; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; mMRC, Modified British Medical Research Council.

Table 3 The relationship between LL-37, 25(OH)D and exacerbation risk in high risk group patients

Variables	LL-37		25(OH)D	
	r	P	r	P
25(OH)D	0.113	0.453	1	
age	0.152	0.307	0.244	0.102
FEV_1%pred	0.018	0.911	0.184	0.243
FEV_1/FVC	−0.127	0.417	0.219	0.163
Hospitalizations for COPD exacerbations per year	−0.290*	0.048	−0.341*	0.020
mMRC score	−0.150	0.527	−0.436	0.062

*, there is correlation between two indexes.

Discussion

COPD is a leading cause of morbidity and mortality worldwide and results in an economic and social burden that is both substantial and increasing (16). Exacerbations of COPD are important events in the course of the disease, particularly in those requiring hospitalization. Until now, the best predictor of having frequent exacerbations is a history of previous treated events (requiring treatment with antibiotics and/or systemic corticosteroids) (17). The objective of our study was to seek new predictors of exacerbation risk in COPD patients. Exacerbations of COPD can be triggered by infection with bacteria or viruses, environmental pollutants, or unknown factors. The most common cause appears to be respiratory tract infection (18,19). Antimicrobial peptides play an important role in innate defense against infection (20).

We measured LL-37, the only human cathelicidin antimicrobial peptide, in COPD patients. We found that plasma levels of LL-37 in COPD patients with high risk of frequent exacerbation were lower than normal subjects. And LL-37 levels inversely associated with the frequency of hospitalization for COPD exacerbations in these high risk patients. Most of the previous studies have focused on the local innate immunity role of LL-37, only a few of them have investigated the circulating levels of LL-37 in COPD patients. To our knowledge, the relationship between circulating levels of LL-37 and

exacerbation risk has not been previously described in patients with COPD. Xiao *et al*. (21) found that hCAP18 levels in induced sputum were elevated in COPD patients compared to control subjects and inversely correlated with pulmonary function. Parameswaran *et al*. (10) reported that sputum LL-37 levels were higher during COPD exacerbation compared with baseline. A small-scale research showed that increased induced sputum levels of LL-37 in COPD patients were associated with airflow limitation, health status and exercise tolerance compared with control group (22). But there were no statistically significant differences in serum LL-37 levels between healthy smokers, healthy non-smokers, COPD patients with FEV_1% more than 50% and less than 50% (22). These results suggested that airway levels of LL-37 are likely to be important in pathogen clearance and clinical outcomes of infection in COPD patients. About the difference between circulating and airway levels of LL-37, we presumed that the increase of airway levels of LL-37 is a local immune response to outside stimulation and plasma levels of LL-37 reflects status of systemic innate immunity. The decline of defence function against infection leads to frequent exacerbations of COPD. The decrease of circulating levels of LL-37 might be increase the risk of exacerbation in COPD patients. Previous research about patients admitted to intensive care units partly confirmed our inference, mean plasma LL-37 levels were significantly lower in critically ill subjects compared to healthy controls (23).

A growing body of evidences suggest an important role for vitamin D in mounting appropriate innate and adaptive immune responses to infections (24). In our study, plasma concentration of 25(OH)D was significantly higher in normal subjects than in COPD patients. The plasma concentrations of 25(OH)D in COPD patients with high risk of frequent exacerbation were significantly lower than in low risk COPD patients. Hospitalization frequency for COPD exacerbations was negative correlated with plasma levels of 25(OH)D in COPD patients with high risk of frequent exacerbation. Previous studies also show that Vitamin D insufficiency and deficiency are highly prevalent in patients with COPD, with the lowest vitamin D levels being associated with the most severe airflow obstruction (12). Quint *et al*. (25) considered that low 25-hydroxyvitamin D levels in COPD were not associated with frequent exacerbations. However, Lehouck *et al*. (26) found that high-dose vitamin D supplementation may reduce exacerbations in patients with severe vitamin D deficiency at baseline. Some previous studies found the ability of $1,25(OH)_2D$ to increase expression of antimicrobial peptides (13,14). In our study, we did not find significant correlation between plasma levels of LL-37 and 25(OH)D. Human cationic antimicrobial protein 18 gene expression, LL-37 peptide release and transformation from 25(OH)D to $1,25(OH)_2D$ were impacted by many factors *in vivo* environment. The regulation of LL-37 levels is a complex process in COPD patients, vitamin D is not the only influencing factor of circulating levels of LL-37.

Based on our data, the plasma levels of LL-37 and 25(OH)D were lower in COPD patients with high risk of frequent exacerbations than normal subjects. Hospitalization frequency for COPD exacerbations was negative correlated with plasma levels of LL-37 and 25(OH)D in high risk COPD patients. So we speculate that low plasma levels of LL-37 and 25(OH)D might be predictors of exacerbation risk in COPD patients, but further large-scale work is necessary to confirm this inference.

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