

## ORIGINAL ARTICLES

# Impact of noninvasive positive pressure ventilation on the gene expression of ubiquitin system of skeletal muscle in patients with acute exacerbation of chronic obstructive pulmonary disease

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Received: September 21, 2016

Accepted: October 21, 2016

Online Published: December 10, 2016

DOI: 10.14725/dcc.v3n4p1

URL: <http://dx.doi.org/10.14725/dcc.v3n4p1>

## Abstract

**Objective:** To investigate the change and relativity of noninvasive positive pressure ventilation (NIPPV) on the gene expression of ubiquitin system of skeletal muscle in patient with acute exacerbation of chronic obstructive pulmonary disease (AECOPD).

**Methods:** 80 patients with AECOPD were divided into two groups based on whether the NIPPV treatment was given or not, 38 cases in the study group and 42 in the control group. The blood gas analysis and pulmonary function were monitored and recorded before and 14 days after treatment. A skeletal muscle biopsy was performed 14 days after therapy. The mRNA expression of ribosomal protein S21 (RPS21) and ubiquitin in skeletal muscle cell were measured by RT-PCR.

**Results:** After 14 days treatment, the levels of PaCO<sub>2</sub>, PaO<sub>2</sub>, PH and FEV<sub>1</sub>% in the NIPPV group improved much better than in the control group ( $p < .05$ ). The gene expression of RPS21 and ubiquitin was obviously lower in the study group than in the control group ( $p < .05$ ). The level of RPS21 was negatively related with PaO<sub>2</sub>, PH and FEV<sub>1</sub>%, and the level of ubiquitin was negatively related with PaO<sub>2</sub> and FEV<sub>1</sub>%, but positively correlated with PaCO<sub>2</sub>. The area under the ROC curves of RPS21, ubiquitin, PaCO<sub>2</sub> and FEV<sub>1</sub>% were 0.771, 0.885, 0.821 and 0.734 respectively in the study group. The cut-off points were 103.978, 8.128, 45.350 and 51.350 respectively. The sensitivity evaluation of acid poisoning was 90.9%, and the specificities for each were 75%, 75%, 50% and 50%.

**Conclusions:** NIPPV is effective for AECOPD patients through the gene expression of ubiquitin system of skeletal muscle.

**Key Words:** Noninvasive positive pressure ventilation, Acute exacerbation of chronic obstructive pulmonary disease, Skeletal muscle, Gene expression

Chronic obstructive pulmonary disease is a common disease in respiratory system. Most of patients with this type of disease also suffer from malnutrition with the symptom of skeletal-muscle wasting and atrophy. The symptom is one of currently recognized extra-pulmonary manifestations and associated with the mortality.<sup>[1,2]</sup> Currently, many researches have confirmed that the degradation of skeletal

muscle protein is closely related to ubiquitin-proteasome pathway.<sup>[3]</sup> However, if patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD) are given treatment with noninvasive positive pressure ventilation (NIPPV) at the appropriate time, it will effectively relieve respiratory muscle fatigue.<sup>[4]</sup> It remains to be further studied whether NIPPV can impede the degradation of

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skeletal muscle protein at the gene level. AECOPD patients given treatment with NIPPV were chosen as subjects in this research. The therapeutic effects of NIPPV on AECOPD patients were further illustrated by studying the blood gas analysis, pulmonary function and the change in skeletal muscle gene expression of AECOPD patients given treatment with NIPPV, as well as relativity among the three factors. The application of ROC curve can further verify that the gene expression of skeletal muscle can be used as a diagnostic assessment of AECOPD acid poisoning.

## 1 Data and methods

### 1.1 Case selection and grouping

70 cases of patients, who were diagnosed as AECOPD by systematic inspections in our department during August of 2012 to October of 2013, were chosen according to the diagnostic criteria in Chronic Obstructive Pulmonary Disease Diagnosis and Treatment Guideline (2013 Revised Edition) established by COPD Group of Chinese Thoracic Society, with indications to NIPPV.<sup>[5]</sup> Patients who had contraindications or relative contraindications to NIPPV were excluded from this study.<sup>[4]</sup> According to the fact that whether patients were willing to receive the treatment with NIPPV or not, the patients were divided into: NIPPV group of 38 cases, given the treatment with NIPPV (S/T mode; parameter: IPAP 14 cmH<sub>2</sub>O, EPAP 4 cmH<sub>2</sub>O), mainly night-time treatment, and the treatment time was more than 14 hours every day; Control group of 42 cases, not given the treatment with NIPPV. Before trial, it was required to acquire consents from the patients and their families with informed consent forms signed.

### 1.2 Clinical observation indicators

The blood gas analysis and the pulmonary function were recorded before treatment and 14 days after treatment.

### 1.3 Muscle biopsy

Vastus lateralis tissues on the left leg were collected by use of biopsy needles (specification: 14 Ga, BARD) from patients with resting state both in the NIPPV group and the control group on the 14<sup>th</sup>-day treatment. Before collection, local anesthesia by use of 2% lidocaine was given to each patient, from which 100 mg muscle tissues were collected and frozen by liquid nitrogen. The samples collected were then placed in the refrigerator with the temperature of -80°C.

### 1.4 Measurement of mRNA expression of ribosomal protein S21 (RPS21) and ubiquitin in skeletal muscle by RT-PCR

Trizol (Gibco, America) reagents were used to extract the skeletal muscle tissues placed at -80°C in the refrigerator. The UV spectrophotometer (Eppendorf, Germany) was applied to the measurement of RNA purity and content. RT-PCR kits (Takara, Dalian) were used in reverse transcription. Gene primers of RPS21 and ubiquitin Ubc were synthesized in Beijing Liuhe BGI Science & Technology Co., Ltd. 5 μL of PCR products were added by 1 μL of loading buffer and mixed together, and then added to the loading well with DNA markers as indicators. After 40-minute electrophoresis, the results were observed and photographed on the FR-200 UV analyzer. The electrophoretic bands were scanned and analyzed by Smart View 2000 bioelectrophoresis image analysis software to calculate integrated optical density (area × optical density). Relative content of gene expression was represented by the ratio of integrated optical density of target gene to that of internal reference GAPDH.

### 1.5 Statistical treatment

The experimental data were analyzed by SPSS 13.0 statistical software and represented by  $\bar{x} \pm s$ . The comparison between specimen means was made by use of *t*-test; the correlation of two variables was determined by linear correlation analysis (Pearson method) to calculate correlation coefficient (*r*), the difference *p* < .05 was of statistical significance. The diagnostic efficacy was analyzed by ROC curve, and the difference *p* < .05 was statistically significant.

## 2 Results

### 2.1 Effects of NIPPV on the blood gas analysis and the pulmonary function of patients with AECOPD

In 14 days after treatment, the levels of PaCO<sub>2</sub>, PaO<sub>2</sub>, pH and FEV<sub>1</sub>% were obviously improved, and the difference (*p* < .05) was statistically significant (see Table 1).

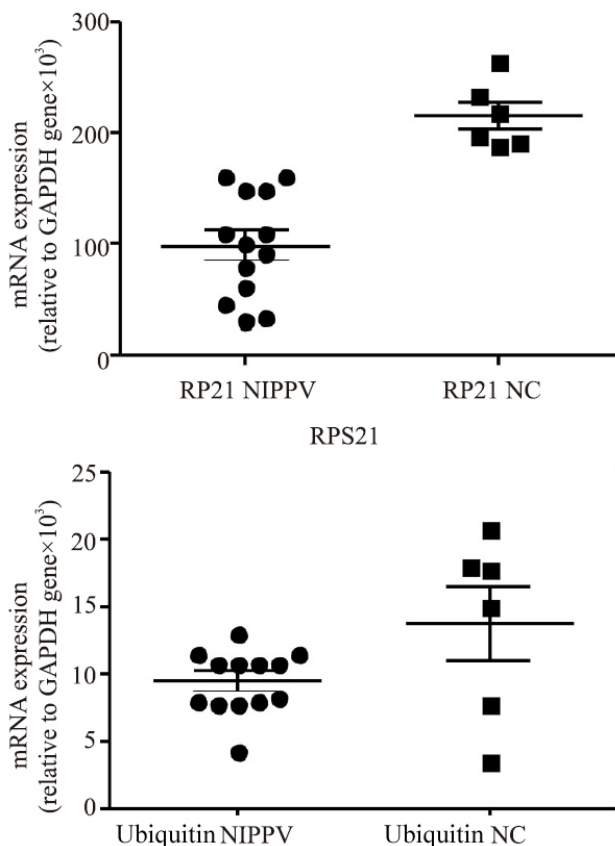
### 2.2 Measurement of mRNA expression of RPS21 and ubiquitin in skeletal muscle by RT-PCR

The mRNA expression of RPS21 and ubiquitin in NIPPV group were: 98.2 ± 45.9 and 9.5 ± 0.9 respectively; the mRNA expression of RPS21 and ubiquitin in skeletal muscle in the control group were: 214.5 ± 28.9 and 13.5 ± 2.8 respectively. The mRNA expression of RPS21 and ubiquitin in skeletal muscle in NIPPV group was obviously lower than that in the control group, *p* < .0001, *p* < .05 (see Figure 1).

**Table 1:** Effects of NIPPV on the blood analysis and the pulmonary function of AECOPD patients ( $\bar{x} \pm s$ )

	NIPPV Group		Control Group	
	Before Treatment	After Treatment	Before Treatment	After Treatment
PaCO <sub>2</sub> (mmHg)	67.1 ± 7.6	52.9 ± 8.1*	65.2 ± 12	60.2 ± 11
PaO <sub>2</sub> (mmHg)	56.8 ± 8.9	77.8 ± 10.4*	55.2 ± 15	69.2 ± 13
pH	7.18 ± 0.15	7.36 ± 0.14*	7.17 ± 0.05	7.35 ± 0.08
FEV <sub>1</sub> %	39.6 ± 5.2	49.1 ± 6.1*	38.2 ± 6.5	47.1 ± 2.3

Note. In comparison with the control group after treatment, \* $p < .05$



**Figure 1:** Comparison of gene expression of RPS21 and ubiquitin in skeletal muscle between NIPPV group and the control group

**2.3 Analysis of correlation between gene expression and the levels of PaCO<sub>2</sub>, PaO<sub>2</sub>, pH and FEV<sub>1</sub>% in skeletal muscle in NIPPV group after 14-day treatment**

The expression of RPS21 was negatively correlated with the levels of PaO<sub>2</sub>, pH and FEV<sub>1</sub>%,  $p < .05$ , and  $r$ -values were -0.8150, -0.7339 and -0.8575 respectively; the expression of RPS21 had no correlation with the level of PaCO<sub>2</sub>,  $p = .2857$ .

The expression of ubiquitin was obviously negatively correlated with the levels of PaO<sub>2</sub> and FEV<sub>1</sub>%,  $p < .05$ ,  $r$ -values

were -0.8565 and -0.8634 respectively; the expression of ubiquitin was positively correlated with the level of PaCO<sub>2</sub>,  $p < .05$ ,  $r = 0.6422$ ; and the expression of ubiquitin had no correlation with the level of pH,  $p = .095$ .

**2.4 AECOPD acid poisoning diagnosed ROC curves of RPS21, ubiquitin, FEV<sub>1</sub>% and PaCO<sub>2</sub>**

The area under the ROC curve of RPS21 was 0.773,  $p = .48$ ; the area under the ROC curve of ubiquitin was 0.875,  $p = .005$ ; the area under the ROC curve of PaCO<sub>2</sub> was 0.813,  $p = .023$ ; and the area under the ROC curve of FEV<sub>1</sub>% was 0.756,  $p = .63$  (see Table 2 and Figure 2).

**3 Discussions**

In recent years, NIPPV has been increasingly applied to the clinical treatment of AECOPD with respiratory failure for its easy-to-operate, non-invasion, effectiveness and other characteristics.<sup>[6]</sup> However, in the process of clinical application, the therapeutic effects of NIPPV were directly affected by various factors and poor treatment compliance (part of patients). Consequently, NIPPV was doubted by part of scholars. Therefore, this study not only explained the therapeutic effects of NIPPV on AECOPD patients from the aspect of the improvement in the blood analysis and the pulmonary function, but also illustrated those effects at the gene level of skeletal muscle. The experimental objective signs and the change in the gene expression of skeletal muscle were indicated and then given correlation analyses.

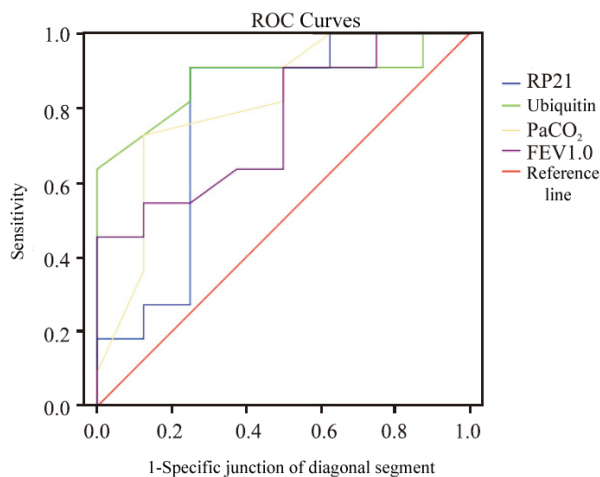
The researches at home and abroad have showed that,<sup>[7-10]</sup> the increase in degradation rate of skeletal muscle protein in AECOPD patients can lead to skeletal-muscle atrophy. However, the process of protein degradation can be divided into 2 steps: at first, with the participation of ATP, ubiquitin-activating enzyme (E1) activates ubiquitin by acting on glutamic acid residue at the end of ubiquitin hydroxy; and then, the activated ubiquitin is bound to L-Cysteine residue of ubiquitin-conjugating enzyme (E2) by use of high-energy sulphhydryl bond.<sup>[11]</sup> Secondly, E2 connects ubiquitin to target protein directly or by use of ubiquitin ligase (E3) to complete the ubiquitination of proteins.<sup>[12]</sup> The gene of RPS21

belongs to the ribosome family. Researches have shown that RPS21 participates in the regulation of protein synthesis and cell growth. Aerobic exercise can lower the expression of RPS21, and then suppress the growth of inflammatory

cells in patients with chronic obstructive pulmonary disease. Meanwhile, it can also lower the gene expression of programmed cell death, and has a positive effect on improving the activity and the lifetime of normal cells.

**Table 2:** The areas under the ROC curves of RPS21, ubiquitin, FEV<sub>1</sub> and PaCO<sub>2</sub> in skeletal muscle in NIPPV group

	Area	p-value	Cut-off Point	Sensitivity (%)	Specificity (%)
RPS21	0.771	.048	103.978	90.9	75
Ubiquitin	0.885	.006	8.128	90.9	75
PaCO <sub>2</sub>	0.821	.023	45.350	90.9	50
FEV <sub>1</sub> %	0.734	.063	51.350	90.9	50



**Figure 2:** The areas under the ROC curves of RPS21, ubiquitin, FEV<sub>1</sub> and PaCO<sub>2</sub> in skeletal muscle in NIPPV group

This research showed that the blood gas analysis and the pulmonary function in NIPPV group of AECOPD patients were obviously improved after treatment in comparison with the control group, the mRNA expression of RPS21 and ubiquitin in skeletal muscle in NIPPV group was obviously lower than that in the control group ( $p < .05$ ). It is indicated that the application of NIPPV can inhibit the degradation of protein in skeletal muscle, partially recover the function of skeletal muscle, suppress the growth of inflammatory cells in AECOPD patients and the gene expression of programmed cell death, and improve the activity of skeletal muscle cells.

The results in this study have shown that the mRNA expression of ubiquitin and RPS21 in skeletal muscle in patients of NIPPV group was lower than that in the control group, and it is negatively correlated with PaO<sub>2</sub> and FEV<sub>1</sub>. It is further indicated that the application of NIPPV treatment can not only improve the function of skeletal muscle through the change in the gene expression, but also make the patient's ventilation condition better in order to realize the therapeutic effects. However, the mRNA expression of ubiquitin has no correlation with pH, and the mRNA expression of RPS21 has no correlation with PaCO<sub>2</sub> either. Considering the limited

number of cases involved in this research and internal related influencing factors, it remains to be further studied.

In the analysis of the ROC curve, it is acknowledged that it is important to select a gold standard. If the gold standard is improperly selected, it will lead to the deviation of results. Currently, in many medical literatures and clinical application, pH value in the blood gas analysis is the most sensitive indicator reflecting whether AECOPD patients suffer from acid poisoning. Meanwhile, this research confirms that pH value has a good correlation with the gene expression of RPS21 in skeletal muscle. With the aid of ROC curves, this research further confirms that the change in mRNA expression of PaCO<sub>2</sub>, ubiquitin and RPS21 is of high sensitivity to the diagnosis of acid poisoning,  $p < .05$ . Ubiquitin has a good correlation with PaCO<sub>2</sub>, and then it is in favor of clinical physicians to make better use of simple and objective indicators derived from experimental results to investigate the internal change of skeletal muscle in AECOPD patients after treatment with NIPPV. However, the analysis of ROC curves in this research has shown that the change in FEV<sub>1</sub> can not be used to determine whether AECOPD patients suffer from acid poisoning. Although the ROC curve is associated with the patient's pulmonary function grading and compensatory mechanism, the correlation with the limited number of cases in this study cannot be excluded. Therefore, it remains to be further studied.

To be concluded, this research can not only confirm the improved AECOPD patients' ventilation conditions after NIPPV treatment according to the blood gas analysis and pulmonary function at the experimental level, but also confirm that NIPPV can impede the degradation of skeletal muscle protein at the gene level and improve patient's ventilation condition, to avoid or relieve the pains caused by tracheal intubation or incision, as well as reduce the incidence of pulmonary infection and its mortality. However, it remains to be further studied that how long NIPPV treatment takes to reverse skeletal-muscle malnutrition.

**Conflicts of Interest Disclosure**

The authors have no conflicts of interest related to this article.

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