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Original Article

The levels of miR-155 and miR-483-5p in the serum of patients are potential diagnostic markers for early COPD

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Abstract: Objective: To study the early diagnostic value of combined detection of miR-155 and miR-483-5p in chronic obstructive pulmonary disease (COPD). Methods: Fifty-three patients with COPD were enrolled for this study as the study group. Another 50 healthy volunteers were enrolled as controls. The relative expression of miR-155 and miR-483-5p in the serum of subjects in the two groups was evaluated by qRT-PCR. Pearson correlation analysis was performed to analyze the association between miR-155 and miR-483-5p in the serum of the study group. The area under the curve (AUC) is used to compare the usefulness of miR-155 and miR-483-5p in diagnosing COPD and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018 was adopted to determine the COPD phases. Results: The expression of miR-155 was significantly lower in the control group than that in the study group ($P<0.05$), while the expression of miR-483-5p was significantly higher in the serum of the control group than that in the serum of the study group ($P<0.05$). Serum miR-155 level was significantly higher in patients during the acute phase than during the stable patients, while serum miR-483-5p level was significantly lower in patients during the acute phase than during the stable phase ($P<0.05$). Pearson correlation analysis found that the relative expression of miR-155 and miR-483-5p in the serum of the experimental group showed negative correlation ($P=0.015$). The area under the ROC curve of miR-155 and miR-483-5p to diagnose COPD was 0.880 and 0.712, respectively. For miR-155 in combination with miR-483-5p, the value was 0.945. Conclusion: The levels of miR-155 and miR-483-5p in the serum of patients with COPD can together serve as potential diagnostic markers for early COPD.

Keywords: miR-155, miR-483-5p, COPD, diagnosis

Introduction

Chronic obstructive pulmonary disease (COPD) is a common type of chronic lung disease with obstructive features. Causes of COPD include abnormalities in the alveoli and airways that are continually exposed to harmful particles in the air, leading to a variety of adverse reactions, including chronic bronchitis and emphysema, which are common presentations of COPD [1]. As a major disease that poses a threat to health and safety, the number of deaths reportedly caused by COPD was 3 million in 2016, accounting for 5.2% of global mortality [2]. In Asian countries, especially in China, more than 1 million people die from COPD each year, and the number of people disabled from it is as high as 5 million [3]. Pathological investigations show [4] the incidence of COPD is as high as 9-10%, mostly in the middle-aged and elderly people aged over 40 years. Such high mortality and disability rates have a serious impact on the quality of life and safety of patients with COPD. Studies have shown that [5] COPD can be effectively prevented by early interventions. Undiagnosed early stage COPD, particularly in symptomatic patients, is likely to progress to more severe stages, with an increased impact on health related quality of life (HRQoL), healthcare costs [6, 7] and greater healthcare resource utilization [8]. Hence, early diagnosis is the key to reduce patient mortality resulting from COPD.

At present, the cause of COPD is still unclear. Most researchers believe that the occurrence of COPD is related to inflammatory responses, oxidative stress, immune mechanisms, genetics, and environmental factors [6]. Recent studies have shown that [7] the occurrence and

Table 1. Primer sequence

Gene	Upstream primer	Downstream primer
miR-155	5'-TTAATGCTAATCGTGATAG-3'	5'-ACCTGAGAGTAGACCAGA-3'
miR-483-5p	5'-AAGACGGGAGGAAAGAAGGGAG-3'	5'-GTGCAGGGTCCGAGGTATTC-3'
U6	5'-CGCTTCGGCAGCACATATACTA-3'	5'-CGCTTCGGCAGCACATATACTA-3'

development of COPD is closely related to molecular mechanisms. MicroRNAs (miRs) are gaining popularity in the field of research in recent years. miRs, expressed in a variety of plants and animals, are endogenous non-coding single-stranded RNA molecules with a length of approximately 22 nucleotides (nt) [8]. The downstream target mRNA can be degraded or inhibited by base pairing with the 3' non-coding region of the target gene to regulate the mRNA [9]. As a typical multifunctional miR, miR-155 is involved in the development of various tumors, such as lung cancer and gastric cancer. Compared with people who never smoke, studies have shown that [10] miR-155 is differentially expressed in COPD patients. miR-483-5p is a miR that has been closely associated with tumors in recent years. Studies have shown that [11] it is differentially expressed in various tumors, such as esophageal cancer and liver cancer, and compared with normal groups it has low expression in COPD patients according to a study by Shen et al. [12]. However, whether miR-155 and miR-483-5p can be used as diagnostic indicators is not clear, and currently there are no relevant diagnostic indicators for early-phase COPD.

Therefore, in this study, we investigated whether the levels of mir-155 and mir-483-5p in the serum of patients with COPD can serve as clinical diagnostic indicators for COPD and aimed to provide a reference range for clinicians.

Materials and methods

In this study, we enrolled 53 patients with COPD, who were treated at our hospital from June 2016 to February 2017, as the study group, which comprised 32 males and 21 females, with the average age being 60.32± 6.35 years (range: 40-69 years). We recruited 50 healthy volunteers who underwent physical examination at our hospital as controls. There were 25 males and 25 females with an average age of 59.83±5.29 years (range: 43-68 years) in the control group. The control group did not use antibiotics, vitamins and hormonal drugs, and they had normal limbs, and normal heart and lung function before the test. This study was approved by the ethics committee of the Hebei Province Hospital of Traditional Chinese Medicine. Patients and their families were informed and signed an informed consent.

Criteria of inclusion and exclusion for patients with COPD

The inclusion criteria were as follows: all patients were older than 40 years; and the patient's clinical data was up to date. All patients in the experimental group met the COPD diagnostic criteria of the Chinese Medical Association Respiratory Branch [13].

The exclusion criteria were as follows: patients with concomitant tumors, congenital defects in the immune system, other structural lesions in the lungs, abnormal heart, liver, and kidney function were excluded.

Main kits and instruments

Trizol extraction reagent (Invitrogen, USA, 15596018), Mir-XTM miRNA qRT-PCR SYBR® Kit (TAKARA, Beijing, China, 638314), and miR-155 and miR181a primers designed and synthesized by Shanghai Shenggong Bioengineering Co., Ltd, as shown in **Table 1**, were used. Mx3000P PCR instrument (Agilent, USA), and ELx808 absorbance microplate reader (BIOTEK, USA) were also used.

Sample collection

Fasting venous blood samples, (~3-5 ml) was collected from patients in both the groups and centrifuged at 5000 rpm, at 4°C for 10 minutes. The upper serum layer of the blood was collected for subsequent experiments. The excess serum was stored in a freezer at -80°C for later use.

PCR detection

Total RNA was extracted from the serum using the Trizol extraction reagent. After extraction, the purity, concentration, and integrity of the

Table 2. Comparison of clinical data between two groups of patients [n (%)]

Factor	Control group (n=50)	Experimental group (n=53)	t/X^2 value	*P* value
Sex			1.121	0.290
Male	25 (50.00)	32 (60.38)		
Female	25 (50.00)	21 (39.62)		
Age (years)	59.83±5.29	60.32±6.35	0.424	0.672
BMI (kg/m^2)	22.18±1.63	21.84±1.55	1.085	0.281
Smoking history			5.566	0.018
Yes	30 (60.00)	43 (81.13)*		
No	20 (40.00)	10 (18.87)*		
History of hypertension			1.165	0.280
Yes	37 (74.00)	34 (64.15)		
No	13 (26.00)	19 (35.84)		
Diabetes history			2.234	0.135
Yes	20 (40.00)	29 (54.72)		
No	30 (60.00)	24 (45.28)		
COPD stage				
Acute stage		23 (43.40)		
Stable period		30 (56.60)		

Note: * indicates that there is a significant difference between the two groups (P<0.05).

RNA were determined by a UV spectrophotometer and gel electrophoresis. The extracted total RNA was reverse transcribed using a reverse transcription kit to obtain cDNA, and the procedure was carried out according to the reverse transcription kit manufacturer's instructions. Two-step PCR amplification was performed using the Mx3000P PCR instrument and a PCR system with 12.5 μL SYBR Advantage Premix (2X), 0.5 μL ROX Dye (50X), 0.5 μL upstream and downstream primers, 2.0 μL cDNA, 0.5 μL miRNA-specific primer (10 μM), made up to a reaction volume of 25 μL with ddH2O. The PCR quantification protocol was as follows: denaturation at 95°C for 10 s, 95°C for 5 s, 60°C for 20 s, for a total of 40 cycles. In this study, U6 was used as an internal reference, and the data was analyzed using 2-ΔΔt. Three replicate wells were set and the experiment was performed three times.

Outcome measures

The relative expression of miR-155 and miR-483-5p in the serum of the two groups and the relative expression of miR-155 and miR-483-5p in the serum of the acute phase and stable phase of COPD were observed. Pearson correlation analysis was used to evaluate the relationship between miR-155 and miR-483-5p in the serum of the study group. The area under the curve (AUC) is used to determine the usefulness of miR-155 and miR-483-5p in diagnosing COPD and their sensitivity, specificity, youden index and cut-off value. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018 was adopted to distinguish COPD phases [14].

Statistical methods

In this study, the collected data were statistically analyzed using the SPSS 20.0 software package (Shanghai Beika), and the data were plotted using GraphPad Prism 7 (Shanghai Beika), in which the enumeration data were expressed as "rate (%)" and represented with the help of the chi-square test. The measurement data was expressed as mean ± standard deviation (mean ± SD), and the measurement data between the two groups in accordance with the normal distribution was analyzed by the independent t test. The correlation between miR-155 and miR-483-5p levels in the serum was analyzed by the Pearson correlation coefficient, and ROC curve was used to assess the sensitivity, specificity and the area under curve. Statistically significant differences were set at P<0.05 (two-tailed).

Results

Comparison of baseline clinical data between the two groups

We analyzed the clinical data of the two groups and found that there was no statistical difference in gender, age, BMI, history of hypertension, and severity of diabetes between the two groups (P>0.05). There was a statistical difference in smoking history (P<0.05) (**Table 2**).

Expression of miR-155 and miR-483-5p in the serum of the two groups of patients

We detected the expression of miR-155 and miR-483-5p in the serum of the two groups by

Table 3. Relative expression of miR-155 miR-483-5p in experimental and control groups

Group	miR-155	miR-483-5p
Control group (n=50)	1.025±0.354	1.013±0.128
Experimental group (n=53)	1.581±0.422*	0.735±0.243*
t value	12.737	7.200
P value	0.000	0.000

Note: * indicates that there is a significant difference between the two groups (P<0.05).

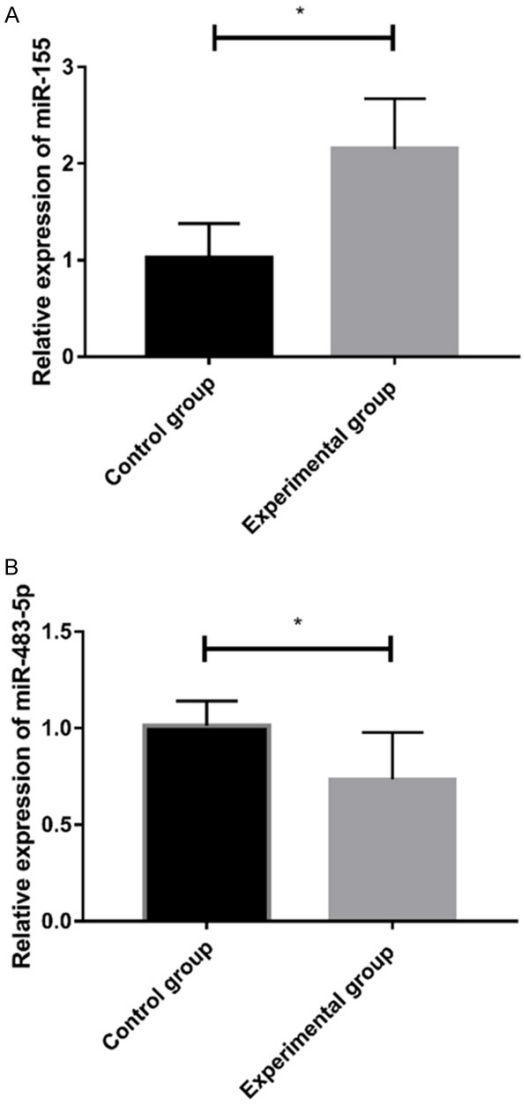

Figure 1. Expression of miR-155 and miR-483-5p in the serum of two groups of patients was detected by qRT-PCR. Expression of miR-155 in the serum of the control group was significantly lower than in the study group. Expression of miR-483-5p in the serum of the control group was significantly higher than in the study group. *Difference between the two groups (P<0.05) was found to be statistically significant.

qRT-PCR. The expression of miR-155 in the serum of the control group was significantly lower than that in the serum of the experimental group (P<0.05). The expression of miR-483-5p was significantly higher in the serum of the control group than in the serum of the experimental group (P<0.05) (**Table 3** and **Figure 1**).

Expression of miR-155 and miR-483-5p in the serum of patients with acute phase and stable phase

We divided the experimental group into acute phase patients and stable patients. The expression of miR-155 and miR-483-5p in the serum of the two groups was determined. The expression of miR-155 was significantly higher in the serum of the patients in the acute phase than in the serum of the patients in the stable phase, while the expression of miR-483-5p was significantly lower in the serum of the patients in the acute phase than in the serum of the patients in the stable phase (P<0.05) (**Table 4** and **Figure 2**).

Correlation analysis of miR-155 and miR-483-5p in the experimental group

We used Pearson correlation analysis to analyze the expression of miR-155 and miR-483-5p in the serum of the experimental group and found that it showed negative correlation (r=-0.332, P=0.015) (**Figure 3**).

ROC curve analysis

We plotted the ROC curve for the expression of miR-155 and miR-483-5p in the serum from both groups and found that the area under the ROC curve of miR-155 was 0.880 (95% CI: 0.808-0.953); sensitivity, 98.11%; specificity, 75.47%; and the Youden index, 73.58%. The area under the ROC curve of miR-483-5p was 0.712 (95% CI: 0.609-0.814); sensitivity, 71.70%; specificity, 75.47%; and Youden index, 47.17%. The ROC curve area of miR-155 in combination with miR-483-5p was 0.945 (95% CI: 0.903-0.987); sensitivity, 84.90%; specificity, 92.45%; and Youden index, 77.36% (**Table 5** and **Figure 4**).

Discussion

COPD is a respiratory disease characterized by airflow limitation that can be prevented and

Table 4. Relative expression of miR-155 miR-483-5p in serum of acute and stable COPD patients

Group	miR-155	miR-483-5p
Acute stage (n=23)	1.873±0.203	0.698±0.187
Stable period (n=30)	1.222±0.213*	0.903±0.171*
t value	11.253	4.154
P value	0.000	0.000

Note: * indicates that there is a significant difference between the two groups (P<0.05).

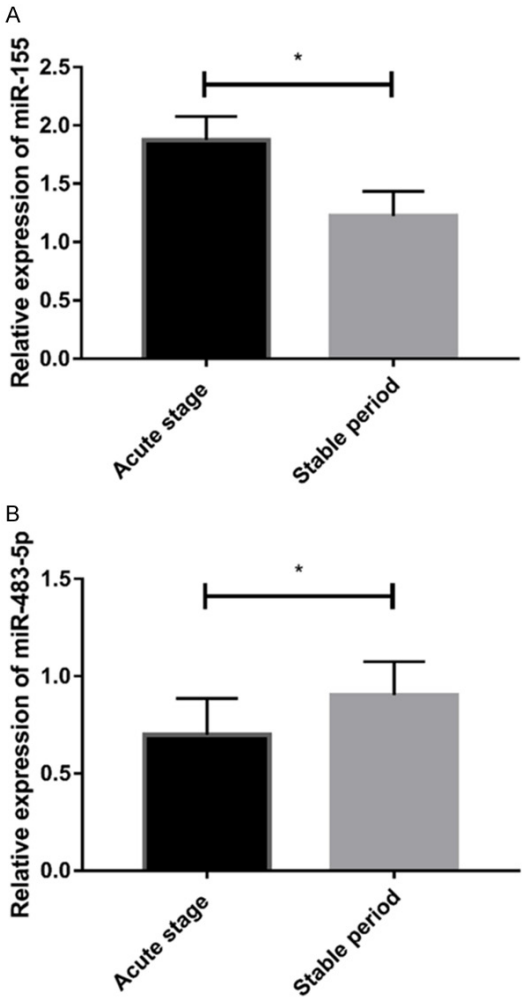

Figure 2. Expression of miR-155 and miR-483-5p in the serum of patients in the acute phase and stable phase was detected by qRT-PCR. The serum miR-155 level in patients in the acute phase was significantly higher than that in patients in the stable phase, while the serum miR-483-5p level in the acute phase patients was significantly lower than that in the stable phase patients, and the difference between the two groups (P<0.05) was found to be statistically significant.

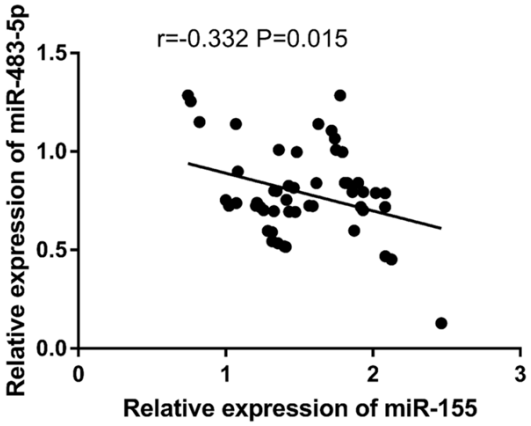

Figure 3. Correlation analysis of miR-155 and miR-483-5p in patients in the study group showed a negative correlation (r=-0.332, P=0.015).

treated. COPD airflow limitation is progressive, and is closely related to the enhancement of chronic inflammatory responses to inhaled particles and hazardous gases in the lungs. COPD mainly causes an inflammatory reaction, not only in the lungs, but may also affect various joints, as well as lead to psychological problems [15]. In recent years, survey statistics show that [16] more than 16 million people have COPD, and it is the fourth leading cause of death in the United States. In China the incidence rate is as high as 8.2% in people over 40 years old, which seriously affects the quality of life of patients [17]. However, the causes and mechanisms of COPD are still unclear. There is no good biological index for diagnosis of COPD, diagnosis is mainly made by presence of limited airflow [18]. Therefore, we hope to find a class of biological indicators which will serve as diagnostic indicators to provide a reference for clinicians.

With the development of molecular biology technology, research on the mechanism and development of COPD has become more and more comprehensive. As a novel molecule, miRs have received extensive attention. miRNA is an endogenous, non-coding, small molecule, single-stranded RNA, with high temporality, conservation and specificity. It can participate in the development of disease by specifically binding to the target gene of interest, and can also participate in the important processes of body cells such as proliferation, apoptosis, growth and tumor development [19]. miR-155

Table 5. ROC curve analysis data

Factor	AUC	Std. Error	95% CI of AUC	Sensitivity %	Specificity %	Youden index	Cut off
miR-155	0.880	0.037	0.808~0.953	98.11%	75.47%	73.58%	1.271
miR-483-5p	0.712	0.052	0.609~0.814	71.70%	75.47%	47.17%	0.628
miR-155/miR-483-5p	0.945	0.021	0.903~0.987	84.90%	92.45%	77.36%	0.978

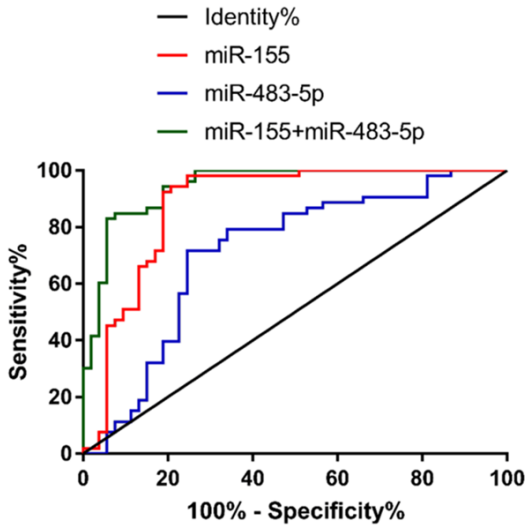

Figure 4. ROC curve analysis. We analyzed the patient with ROC curve analysis. The area under the ROC curve of miR-155 was 0.880 (95% CI: 0.808-0.953); sensitivity, 98.11%; specificity, 75.47%; and Jordan index, 73.58%. The area under the ROC curve of miR-483-5p was 0.712 (95% CI: 0.609-0.814); sensitivity, 71.70%; specificity, 75.47%; and Jordan index, 47.17%. The area under the ROC curve of miR-155 in combination with miR-483-5p was 0.945 (95% CI: 0.903-0.987); sensitivity, 84.90%; specificity, 92.45%; and the Youden index, 77.36%.

is located in the third exon of BIC, a non-coding transcript of human chromosome 21, and studies have shown that [19] miR-155 has a role in tumor formation, inflammation, immunity, viral infections, and cardiovascular diseases. In this study, the expression of miR-155 was found to be significantly lower in the serum of the control group than in the study group. Literature shows that [20] LSP can increase the expression of miR-155 by inducing monocytes. Monocytes are important cells that secrete inflammatory factors and can promote the expression of TNF-α and IL-6, while the exacerbation of COPD is mainly related to the inflammatory mechanism. Therefore, we speculate that miR-155 is closely associated with the occurrence and development of COPD. The study by Kara M [21] reported that miR-155 is highly expressed in COPD patients, which is consistent with our findings. miR-483-5p has become a popular member of miR in recent years. Studies have shown that [22] miR-483-5p is differentially expressed in various tumors, liver cancer, and glioblastoma. We found that the expression of miR-483-5p in serum of patients with COPD was significantly lower than that of the control group. The study showed [23] that miR-483-5p can target the regulation of ERK1 protein expression. The ERK1/2 pathway induces the expression of inflammatory factors, which suggests that differential expression of miR-483-5p may be associated with the development of COPD.

Moreover, we analyzed the expression of miR-155 and miR-483-5p in the serum of patients in the acute phase and stable phase in the study group, and found that the expression of miR-155 in the serum of patients in the stable phase was significantly lower than that in the acute phase, while the expression of miR-483-5p in the serum of patients in the stable phase was significantly higher than that of patients in the acute phase, which indicated that the expression of miR-155 and miR-483-5p is associated with the progression of COPD. We also analyzed the correlation between the expression of miR-155 and miR-483-5p in the study group, and found a negative correlation. There may in fact be a regulatory relationship between them, which needs to be verified by further research. At the end of the study, we performed ROC curves on the expression of miR-155 and miR-483-5p, and found that the areas under the curve of miR-155 and miR-483-5p were 0.880 and 0.712 respectively, and they were highly sensitive and specific. However, we found that the AUC was 0.945 through combined detection, which was significantly higher than that of the single detection. The sensitivity and specificity are also better than in single detection, thus indicating that combined detection has a good predictive value in the diagnosis of COPD.

In recent years, with the increasing incidence of COPD, the treatment of COPD has gradually received attention. Although no drugs can

reverse the decline of lung function in COPD at present [24], the quality of life of patients has significantly improved through effective drug therapy. Moreover, studies have shown that [25] prevention along with timely detection and treatment of patients through early diagnosis can slow down their progress. We have demonstrated through this experiment that the detection of miR-155 and miR-483-5p in the serum of patients has potential diagnostic value for COPD, but there are still some limitations of this study. First, we have a small sample size. Second, there is no comprehensive study of the mechanism of miR-155, miR-483-5p in COPD. We hope to increase our sample size in future studies, and improve basic research to explore the specific mechanism of miR-155 and miR-483-5p in COPD, to verify the results of our study.

In this study, we detected the serum miR-155 and miR-483-5p in normal people and COPD patients, and found significant differences. In conclusion, the combined detection of miR-155 and miR-483-5p in the serum of patients with COPD can serve as a potential diagnostic marker for early COPD.

Disclosure of conflict of interest

None.

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