European Review for Medical and Pharmacological Sciences 2016; 20: 4756-4760

# The expression research of miR-210 in the elderly patients with COPD combined with ischemic stroke

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**Abstract. – OBJECTIVE: We conduct this study to investigate the expression level of miR-210 in elderly patients with COPD combined with ischemic stroke and analyzed its application value as a sensitive diagnostic indicator.**

**PATIENTS AND METHODS: 50 cases of elderly patients with COPD combined with ischemic stroke were selected as group A, 50 cases of elderly patients with COPD were selected as group B, 50 cases of elderly patients with ischemic stroke were selected as group C, and 50 cases of healthy volunteers as group D. Real-time PCR assay for quantification was used to detect the expression level of miR-210 in peripheral blood and receiver operating curve (ROC) was used to analyze the diagnostic sensitivity and accuracy.**

**RESULTS: MiR-210 level of group A was the lowest, followed by group B and group C; group D had the highest levels. The difference was statistically significant ($p<0.05$). MiR-210 levels decreased with an increasing decline degree of lung function and the difference was statistically significant ($p<0.05$). After miR-210 diagnosis, COPD sensitivity was 85.6%, the specificity was 72.6%, accuracy (AUC) was 0.821, and 95% CI 0.632-0.924.**

**CONCLUSIONS: The down-regulation of MiR-210 expression in the COPD combined with ischemic stroke can be regarded as a sensitive index in diagnosis of COPD and ischemic stroke.**

Key Words:
miR-210, COPD, Ischemic stroke, Receiver operating curve.

## Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most common respiratory diseases. 10-15% of all elderly people have COPD; this disease eventually progresses to respiratory failure and heart failure[1]. Cerebrovascular disease has become the leading disease that threatens human health, with characteristics of high incidence, high disability rate and high mortality, of which 70-85% is due to ischemic stroke[2]. According to statistics, there are about 2-3.5 million new cases of COPD combined with ischemic stroke, with the mortality rate of about 30-55%[3]. COPD and ischemic stroke are multifactorial, polygenic chronic diseases, which work manifest under combined actions of genetics and the environment. MicroRNAs (miRNAs) play an important role in regulating the occurrence and development of disease and may become an important target for prevention and treatment of diseases[4]. There are more than 70 kinds of miRNAs that have abnormal expressions in the circulating blood of the COPD patients that have been detected through gene sequencing technology[5]. It can be concluded from the animal models of ischemic stroke and clinical research that about 89 miRNAs expression were significantly down-regulated and 35 were up-regulated, which showed certain changes with the progression of the disease[6]. Among many different miRNAs, miR-210 is the most stable and significant. MiR-210 is closely related to various respiratory diseases, including pneumonia, pulmonary fibrosis, asthma, COPD, pulmonary tuberculosis and tumors[7]. It is confirmed in the animal model of ischemic stroke that under the absence of blood or oxygen condition, miR-210 can participate in the regulation of cell proliferation, cell cycle, angiogenesis induction as well as nerve regeneration promotion and so on. Based on this, the study aims to analyze the expression levels of mir-210 in the elderly COPD patients combined with ischemic stroke, and to analyze its application value as a sensitive diagnostic index.


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## Patients and Methods

### Patients

50 cases of elderly patients with COPD combined with ischemic stroke that were admitted to our hospital from January 2015 to January 2016 were continuously selected as group A, 50 cases of elderly patients with COPD alone were selected as group B, 50 cases of patients with ischemic stroke as group C and 50 cases of healthy volunteers as group D. Inclusion criteria: 1. COPD was at acute and stable period, ischemic stroke at acute period; 2. Ages range from 65-80 years old; 3. Data was collected before admission and treatment, and were complete and analyzable. Exclusion criteria: 1. The presence of other lungs disease, such as lung cancer, pneumonia, asthma, respiratory failure, etc.; 2. ischemic stroke was at stable period, with other brain diseases, such as hemorrhagic stroke, brain tumor, encephalitis, etc; 3. The presence of other underlying diseases, such as heart, liver, kidney and other organs dysfunction, autoimmune disease, systemic infectious disease, infectious disease, etc.

Our study obtained the approval of the Ethics Committee of our hospital and the informed consent of patients and their families. Among them, 26 patients were male and 24 patients were females in group A. Patients ages ranged from 66-78 years old with an average of (72.3±10.2) years old. The course of COPD was 1-8 years, with an average of (4.2±2.5) years. 24 cases of COPD were at acute period and 26 cases at stable period. The course of the ischemic stroke was 0.5-3h with an average of (1.3±0.6) h; this one was combined with hypertension in 12 cases, diabetes in 6 cases, smoking in 20 cases. 25 patients were males and 25 patients were females in group B; ages ranged from 68-79 years old with an average of (73.5±12.4) years old. The course of COPD in group B was 2-9 years with an average of (4.6±2.4) years; 23 cases of COPD were at acute period, 27 cases at stable period. The disease = combined with hypertension in 13 cases, diabetes in 5 cases and smoking in 22 cases. 24 cases were males and 26 cases were females in group C and ages ranged from 68-80 years old with an average of (73.6±12.5) years old; the course of ischemic stroke was 1.0-3.5h with an average of (1.6±0.8) h. This was combined with hypertension in 14 cases, diabetes in 8 cases and smoking in 16 cases. 26 patients were males and 24 patients were females in group D; ages ranged from 67-80 years old with an average of (73.8±11.8) years old. The disease was combined with hypertension in 16 cases, diabetes mellitus in 7 cases and smoking in 19 cases. The baseline data between groups were comparable.

### Methods

The Real-time quantitative PCR was used to detect the expression levels of mir-210 in peripheral blood and the Japanese HI-801 portable lung function instrument was employed to detect the lung function of the patients in stable condition.

The main instruments of PCR method: 9600 type PCR amplification instrument (ABI, Vernon, CA, USA), RT-PCR detector (Bio-Rad, Hercules, CA, USA), BCM-8 ultra clean working table (Liuyi Instrument Factory, Beijing, China), micropipette (Eppendorf, Hamburg, Germany).

The main steps included: obtain 5 ml of peripheral venous blood of, centrifuged at 3000 g for 20 minutes and stored at -20°C for detection. AM1556 kit provided by the Invitrogen (Carlsbad, CA, USA) was applied to extract total RNA. Ultraviolet spectrophotometer (BioTek, Winooski, VT, USA) was used to determine the concentration of RNA and a 1% agarose gel electrophoresis was used to determine the integrity of RNA. TaqMan MicroRNA Reverse Transcription kit supplied by Media Cybernetics Company of the United States was used to synthesize cDNA, primer design (Applied Biosystems, Foster City, CA, USA): miR-210 (F): 5'- TGCGGCTGTGCGTGTGACAG-3', (R): 5'-CCAGTGCAGGGTCCGAGGT-3'; hsa-miR-16 mouse was applied as a reference gene. The reaction system: 1:20RT. Product: cDN5.0 μL+ 5 pmol/μL. PCR positive and negative primers: 0.5 μL+ 2×SYBR Green PCR master mix: 10 L and water was added to 20 L. Reaction conditions: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 15s, annealing at 65°C for 15s, extension 32s, a total of 40 cycles; melting curve was used to analyze the temperature of 60°C-95°C. The results were expressed as the ratio of the target gene and reference gene through $2^{-\Delta\Delta Ct}$ method.

### Statistical Analysis

SPSS20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data was expressed by the mean ± standard deviation. Comparisons among groups were analyzed by One-way ANOVA. Comparison between any two groups was tested by the LSD method. Count data were expressed by rate. Comparisons among groups were tested by $\chi^2$ and sensitivity and accuracy of miR-210 were analy-

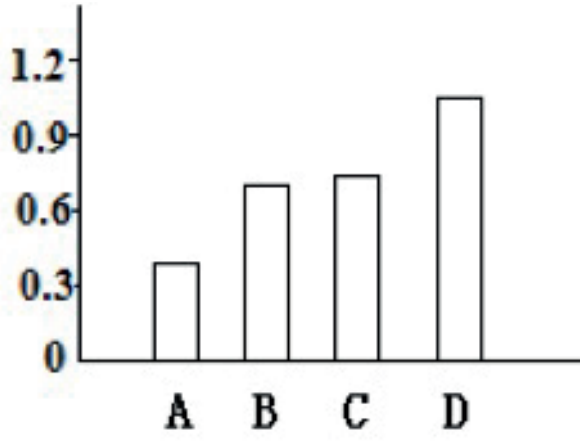


**Figure 1.** Comparison of mir-210 levels between groups (group A, elderly patients with COPD combined with ischemic stroke; group B, elderly patients with COPD; group C, patients with ischemic stroke; group D, healthy volunteers).

zed by receiver operating curve (ROC). $p<0.05$ indicated that the difference was statistically significant.

## Results

### Comparison of miR-210 Levels Between Groups

The levels of miR-210 in group A were the lowest, followed by those of group B and group C; the levels of group D were the highest. The difference was statistically significant ($p<0.05$) (Figure 1).

The lung function results of 100 cases with COPD showed that there were 18 cases with $FEV_1/FVC$ >0.7 and $FEV_1$ ≥0.8, suggesting a lung function decline tendency. A total of 35 cases with $FEV_1/FVC$ ≤0.7, $FEV_1$ ≥0.8 suggested mild pulmonary dysfunction. A total of 32 cases with $FEV_1/FVC$ ≤0.7, $FEV_1$ in 0.5-0.8 suggested a moderate decline in lung function. A total of 10 cases with FEV1/FVC ≤0.7, $FEV_1$ in 0.3-0.5, suggested a severe pulmonary dysfunction as well as a total of 5 cases with $FEV_1/FVC$ ≤0.7, $FEV_1$ <0.3, which suggested especially severe lung function decline. MiR-210 levels decreased with increasing decline in the degree of the lung function, and the difference was statistically significant ($p<0.05$) (Figure 2).

### ROC Curving Analysis

Using COPD as the diagnostic criteria, it was derived from the use of ROC curve that the diagnostic sensitivity of miR-210 was 85.6%, specificity was 72.6%, accuracy (under the curve area) was 0.821, 95% CI was 0.632-0.924. Using ischemic stroke as diagnostic criteria, the diagnostic sensitivity of miR-210 was 81.2%, specificity was 78.3%, accuracy was 0.802, 95% CI was 0.635-0.945 (Figure 3).

## Discussion

miRNAs can combine with their target mRNA 3' untranslated region, which transcriptionally silences the gene and plays an important role in development, cell differentiation, proliferation and apoptosis[8]. The occurrence of COPD is closely related to environmental factors, such as pollution and smoking. It has been confirmed that [9-11] the downregulation of miR-210 expression in patients with COPD increases the expression of PGE2, with the induction of hypoxia inducible factor-1α (HIF-1α)[12]. p53 gene expression increase and miR-210 expression decreases, which positively correlates the deactivation of AKT[13]. The p53 gene regulates the miR-210 expression in response to DNA damage and rehabilitates the DNA repair process[14]. It was concluded from the study that miR-210 levels of the group of patients with COPD combined with ischemic stroke was the lowest, followed by patients with COPD alone and then patients in the ischemic stroke group; levels of healthy volunteers was the highest. The differences were statistically significant. Through further analysis, it was seen that miR-210 levels

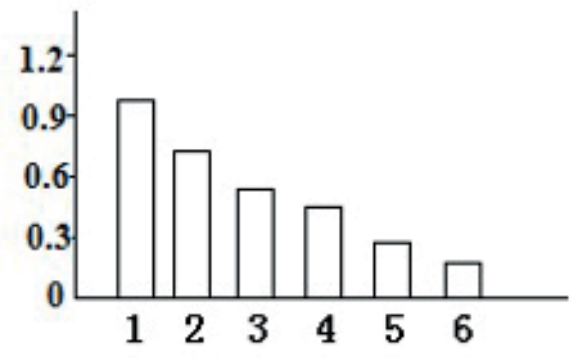


**Figure 2.** Comparison of miR-210 levels of different lung function (1 refers to the group C and D group, 2 refers to the lung function decline tendency, 3 refers to mild pulmonary function decline, 4 refers to moderate pulmonary function decline, 5 refers to severe pulmonary function decline, 6 refers to the special severe pulmonary function decline).

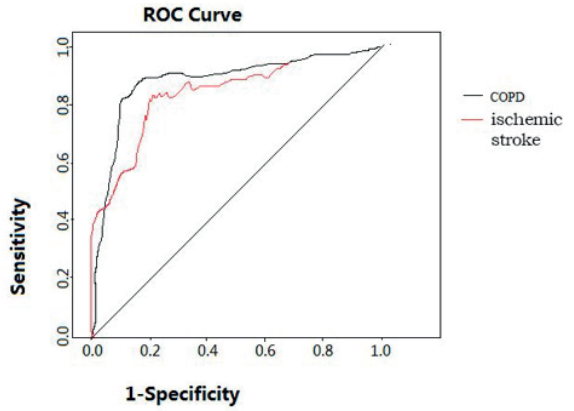


**Figure 3.** ROC analysis of COPD combined with ischemic stroke with miR-210 diagnosis.

decreased with an increasing decline in the degree of pulmonary function, and the difference was statistically significant. It has been derived from the analysis of ROC that through miR-210 diagnosis, the severity of COPD was 85.6%, the specificity 72.6%, and the accuracy was 0.821. It has been confirmed that downregulation of MiR-210 expression in the COPD combined with ischemic stroke was related to the severity of patients' condition and can be regarded as a sensitive index in diagnosis of COPD and ischemic stroke.

In the process of the occurrence, development and nerve repair of ischemic stroke, miR-210 regulation of cell proliferation and cell cycle can manifest through the downregulation of cell proliferation protein expression. In the process of DNA replication, E2F3 regulates cell cycle to transition cells from G1 to S phase, promoting the cell proliferation[15]; E2F3 is the direct target of miRNA-210. In addition, FGFRL1 and HOXAL are the protein targets of miR-210; excessive activation of HOXAL can activate p44/42map kinase, which can promote cell proliferation[16]. MiR-210 can downregulate the expression of cysteine protease 8 which promotes apoptosis (Caspase 8) and increases the survival of mesenchymal stem cells[17]. MiR-210 induces angiogenesis, which depends on the expression of the Dicer gene, and is a key regulatory factor in the survival and migration of vascular endothelial cells [18]. MiR-210 is overexpressed in normal vascular endothelial cells and promotes angiogenesis through the downregulation of the expression of the target gene ephrinA3[19]. It has been confirmed that the expression levels of miR-210 were correlated to the severity of ischemic stroke[20]. The promoting nerve regeneration mechanism of miR-210 is to inhibit neuronal apoptosis and to regulate the balance of Bcl-2 and Bax by retraining the activity of caspase[21].

## Conclusions

It has been derived from this study that the levels of miR-210 in the groups of COPD combined with ischemic stroke were lower than those of the groups of COPD alone and ischemic stroke alone, suggesting that miR-210 is related to COPD and ischemic stroke incidence. It has been concluded from ROC analysis that, through miR-210 diagnosis, the ischemic stroke sensitivity is 81.2%, specificity is 78.3% and accuracy is 0.802. These results suggest that the downregulation of miR-210 in ischemic stroke can be used as a sensitive index in diagnosis of COPD and ischemic stroke.

### Conflicts of interest

The authors declare no conflicts of interest.

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