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Real-time PCR technique for diagnosing respiratory diseases: miR-155, miR-17, miR- 181 Expression and COPD

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Abstract

Background and Objective:

Chronic Obstructive Pulmonary Disease (COPD) is a progressive inflammatory condition with limited non-invasive biomarkers for diagnosis and monitoring. Circulating microRNAs (miRNAs), due to their involvement in inflammation, oxidative stress, and tissue remodeling, offer potential as molecular biomarkers. This study aimed to compare the expression of miR-155, miR-17, and miR-181 in COPD patients versus healthy individuals to assess their diagnostic value.

Methods

This cross-sectional study enrolled 30 COPD patients and 30 age- and sex-matched healthy controls. Peripheral blood samples were collected in EDTA tubes, and total RNA was extracted using the RNeasy Midi Kit (Qiagen). cDNA synthesis was performed using the ZIST ROYESH kit, and expression levels were quantified by SYBR Green-based real-time PCR. U6 snRNA served as the reference gene, and expression was calculated using the 2^-ΔΔCt method. Ct ≤ 35 was considered positive.

Results

miR-181 and miR-155 were significantly upregulated in COPD patients with 2.18-fold and 2.32-fold increases, respectively ($p < 0.001$). Positive expression was detected in 83.3% and 66.7% of patients, respectively. In contrast, miR-17 was downregulated (0.49-fold; $p < 0.001$) and positive in only 23.3% of patients compared to 80% of controls. No significant differences in age or sex were observed between groups.

Conclusion

Distinct expression profiles of miR-155, miR-17, and miR-181 were observed in COPD patients, highlighting their potential as non-invasive biomarkers. miR-181 showed the highest diagnostic sensitivity, while miR-17 downregulation may reflect remodeling activity. Combined miRNA profiling could enhance COPD diagnosis and stratification.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a progressive respiratory disease characterized by persistent airflow limitation associated with an enhanced chronic inflammatory response in the airways and the lung [1–3]. Although it is considered a preventable/treatable disease, it has now become a major concern for global health [2]. The World Health Organization (WHO) anticipates that by 2030, COPD will rank as the third leading cause of mortality worldwide [4].

Despite advancements in understanding COPD pathogenesis, effective biomarkers for early diagnosis, disease progression, and exacerbation risk assessment are still lacking [5]. MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression post-transcriptionally. They have been implicated in various biological processes, including inflammation, immune response, and tissue remodeling, all of which are crucial in COPD pathogenesis [6–8]. Circulating miRNAs, detectable in blood and other body fluids, hold promise as non-invasive biomarkers for various diseases, including COPD.[9]

Among candidate miRNAs, miR-155 emerges as a master regulator of neutrophilic inflammation through NF-κB activation [10–12], while miR-181 modulates oxidative stress responses [13, 14]. Conversely, miR-17 demonstrates context-dependent roles in TGF-β signaling and tissue remodeling [15–17]. This study provides the first comparative analysis of all three miRNAs within the same COPD cohort, enabling direct evaluation of their relative diagnostic values and pathogenetic contributions.

2. Materials and methods

2.1. Study Design and Participants

This cross-sectional study enrolled 30 COPD patients and 30 age- and sex-matched healthy controls from Masih Daneshvari Hospital (2023–2024). Participants were assessed for the expression of miR-181, miR-17, and miR-155.

COPD patients were included based on respiratory symptoms (persistent dry cough, fever, wheezing, shortness of breath, and sputum) and GOLD criteria [1] diagnosis. Healthy controls had no history of respiratory diseases. Exclusion criteria included asthma, allergies, tuberculosis, and current respiratory infections.

2.2. Sample Collection and RNA Extraction

Peripheral blood samples (3 mL) were collected in EDTA tubes and immediately processed. Total RNA was isolated using the RNeasy Midi Kit (Qiagen) following the manufacturer's protocol. RNA purity was assessed by 260/280 nm absorbance ratio, with ~ 2.0 considered acceptable for further analysis.

2.3. cDNA Synthesis and Real-Time PCR Analysis

Complementary DNA (cDNA) synthesis was performed in triplicate using the ZIST ROYESH Kit. Real-time PCR analysis was conducted on an ABI 7300 RT-PCR machine (Applied Biosystems, USA) using SYBR Green Master Mix. U6 small nuclear RNA (snRNA) served as the housekeeping gene. Primers were designed using AlleleID7 software. Cycling conditions were: 95°C (15 min), followed by 40 cycles of 95°C (15 sec)/60°C (60 sec), and melting curve analysis (65–95°C).

2.4. Ethical Consideration

This study was conducted in accordance with the Declaration of Helsinki principles and approved by the Institutional Review Board of the National Research Institute of Tuberculosis and Lung Diseases

(NRITLD) (**IR.SBMU.NRITLD.REC.1403.059**). All participants provided written informed consent. Data confidentiality and participant privacy were ensured through anonymization and secure storage. Participants were informed of their right to withdraw without consequences. Trained medical professionals collected samples following appropriate safety protocols.

2.5. Statistical Analysis

Data were analyzed using SPSS version 22 (IBM Corp., Armonk, NY, USA). Demographic comparisons used independent t-tests for continuous variables and chi-square tests for categorical variables. MiRNA expression levels were calculated using the 2^-ΔΔCt method. Statistical significance was determined using ANOVA test ($p < 0.05$). Positivity was defined as $Ct \leq 35$, with significance thresholds of ≥ 2.0-fold upregulation or ≤ 0.5-fold downregulation ($p < 0.001$).

3. Results

3.1. Demographic Data

There was no statistically significant difference between the COPD and healthy groups regarding gender and age (p-value > 0.05), indicating that gender and age did not affect the miRNA expression results.

1. **MiRNA Expression Analysis**
2. miR-181 Expression:

miR-181 was significantly upregulated in COPD patients. Positive expression was observed in 25/30 (83.3%) COPD patients versus 5/30 (16.7%) healthy controls ($p < 0.001$) (Table 1) (Fig. 1, a). The fold change in expression was 2.18 times higher in COPD patients. (Fig. 1, d)

2. miR-17 Expression:

miR-17 was downregulated in COPD patients. Positive expression was detected in 7/30 (23.3%) COPD patients compared to 24/30 (80%) healthy controls ($p < 0.001$) (Table 1) (Fig. 1, b). The expression in COPD patients was 0.49 times (2.03-fold decrease) that of healthy subjects. (Fig. 1, e)

3. miR-155 Expression:

mmiR-155 was significantly upregulated in COPD patients compared to healthy controls. Positive expression was observed in 20/30 (66.7%) COPD patients versus 4/30 (13.3%) healthy controls ($p < 0.001$) (Table 1) (Fig. 1, c). The fold change in expression was 2.32 times higher in COPD patients. (Fig. 1, f)

Table 1
Comparative analysis of miRNA expression in COPD patients and healthy controls

miRNA	Expression in COPD	Fold Change	Positivity (COPD/Control)	p-value
miR-181	Upregulated	2.18×	25/30 (83.3%) vs. 5/30 (16.7%)	< 0.001
miR-17	Downregulated	0.49× (↓2.03×)	7/30 (23.3%) vs. 24/30 (80%)	< 0.001
miR-155	Upregulated	2.32×	20/30 (66.7%) vs. 4/30 (13.3%)	< 0.001

These results suggest that miR-181 and miR-155 are significantly upregulated in COPD patients, while miR-17 is downregulated, potentially serving as biomarkers for COPD.

4. Discussion

Our investigation of circulating miRNA profiles in COPD patients reveals significant alterations in miR-181, miR-17 and miR-155 expression patterns that both corroborate and extend current understanding of their roles in pulmonary pathophysiology. These findings contribute to the growing body of evidence supporting the involvement of miRNA-mediated gene regulation in COPD pathogenesis while highlighting important novel insights in their disease-specific behavior.

1. miR-155: Inflammation Amplifier

The significant upregulation of miR-155 in COPD patients (2.32-fold increase, $p < 0.001$) strongly affirms its role as a key regulator of pulmonary inflammation. **This pro-inflammatory miRNA has been mechanistically linked to NF-κB pathway activation and subsequent elevation of cytokines including TNF-α and IL-8** [10–12], **which collectively drive the characteristic neutrophilic inflammation and tissue remodeling seen in COPD.** This aligns with De Smet et al.[18], who demonstrated miR-155 upregulation in both COPD patients and cigarette smoke (CS)-exposed mice, our results further solidify the association between miR-155 and CS-induced inflammation.

2. miR-181 Paradox

Our findings of significantly elevated miR-181 levels in COPD patients present an intriguing contrast to its known anti-inflammatory actions within lung tissue. This apparent contradiction underscores the context-dependent nature of miRNA regulation in COPD pathogenesis. While circulating miR-181 is elevated in association with disease severity and exacerbations [19], studies have demonstrated its protective, anti-inflammatory role within lung tissue, where it suppresses HMGB1/CCN1-mediated NF-κB signaling [13, 14]. The elevated levels in circulation may represent a compensatory response to chronic inflammation or a contribution to disease progression through oxidative stress and altered glucocorticoid sensitivity.

miR-17: Divergent Roles in Pulmonary Diseases

The downregulation of miR-17 in COPD patients highlights its context-dependent roles in different pulmonary diseases. As part of the miR-17 ~ 92 cluster, miR-17 exhibits opposing effects in COPD and pulmonary arterial hypertension (PAH)—suppressing fibrosis in COPD while promoting vascular remodeling in PAH [20]. This suggests miR-17's function is shaped by disease-specific microenvironments. In COPD, reduced miR-17 may protect against TGF-β-driven fibrosis and airway remodeling, potentially slowing disease progression [17]. The contrasting expression patterns in COPD (downregulated) and PAH (upregulated) emphasize miR-17's pleiotropic nature in respiratory diseases.

Clinical Translation

The high miR-181 positivity (83.3%) suggests superior diagnostic sensitivity versus miR-155 (66.7%), while miR-17's inverse pattern (23.3%) could indicate disease progression. As Patra et al. demonstrated for spirometry [3], combining miRNA profiling with conventional tests may improve early detection in high-risk smokers. The stability of EV-encapsulated miRNAs [9] supports their clinical utility, though standardization challenges remain.

Therapeutic Horizons

Zhang et al.'s PAH findings [20] and our COPD data reveal miR-17's pleiotropic effects, suggesting pathway-specific targeting will be essential. Anti-miR-155 therapies show promise based on murine models [18], while miR-181 modulation might address glucocorticoid resistance [14]. Future trials should stratify patients by miRNA profiles to assess personalized treatment responses.

5. Conclusion

Our study establishes distinct expression signatures for miR-155, miR-17, and miR-181 in COPD patients, with miR-181 showing particular diagnostic promise. These miRNAs map to different pathogenic axes - inflammation (miR-155), oxidative stress (miR-181), and remodeling (miR-17) - suggesting their combined analysis could provide a multidimensional assessment of COPD activity.

Abbreviations

COPD
Chronic Obstructive Pulmonary Disease
miRNA
microRNA
PCR
Polymerase Chain Reaction
Ct
Cycle Threshold

snRNA
Small Nuclear RNA
NF-κB
Nuclear Factor kappa-light-chain-enhancer of activated B cells
TGF-β
Transforming Growth Factor-beta
EV
Extracellular Vesicle
GOLD
Global Initiative for Chronic Obstructive Lung Disease
RT-PCR
Real-Time Polymerase Chain Reaction
2^-ΔΔCt
Relative Quantification Method
SPSS
Statistical Package for the Social Sciences

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the National Research Institute of Tuberculosis and Lung Diseases (NRITLD) (Approval Code: IR.SBMU.NRITLD.REC.1403.059). Written informed consent was obtained from all participants prior to enrollment.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no known competing financial or non-financial interests that could have appeared to influence the work reported in this paper.

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Final approval of the manuscript: All authors read and approved the final manuscript.

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Figures

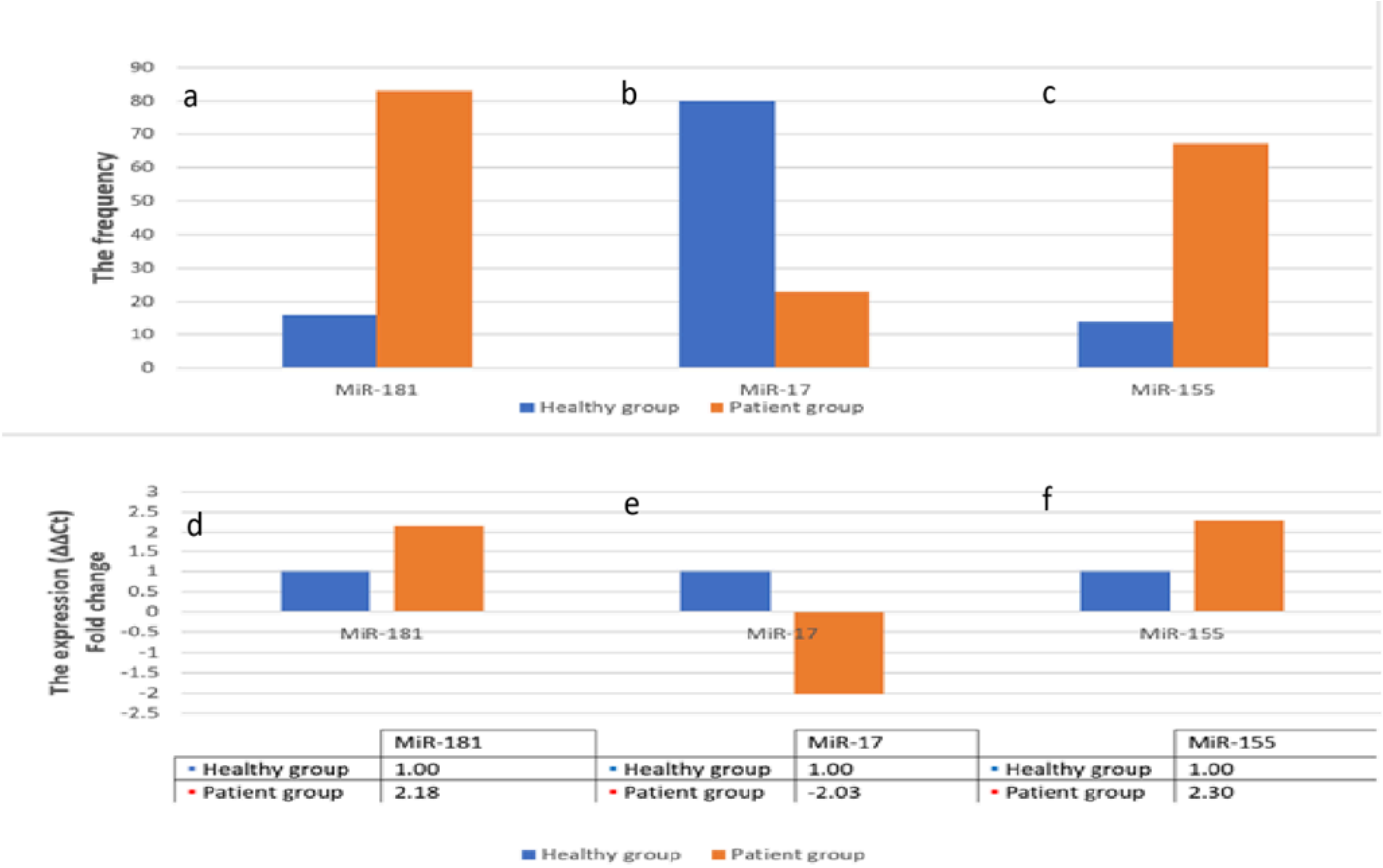

Figure 1

The frequency: (a) miR 181, (b) miR 17, and (c) miR 155 in chronic obstructive pulmonary disease patients and healthy subjects as well as the expression level (using 2−ΔΔct): (d) miR 181, (e) miR 17, and (f) miR 155 in COPD patients and healthy subjects.