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Evaluation of miR-155 Expression in Peripheral Blood Samples of COPD Patients

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ABSTRACT

Introduction: Chronic obstructive pulmonary disease (COPD) is a progressive, irreversible chronic inflammatory disorder characterized by the increased recruitment of monocytes, lymphocytes, and neutrophils. Beyond the lungs, COPD is associated with systemic inflammation, skeletal complications such as osteoporosis, and poor oral and periodontal health, all of which are clinically relevant in oral and maxillofacial practice. This study aims to investigate the changes in miR-155 expression in the peripheral blood of COPD patients compared to healthy individuals.

Materials and Methods: In this descriptive-cross-sectional study, 35 peripheral blood samples from COPD patients and 35 peripheral blood samples from healthy individuals were collected. RNA extraction was immediately performed, followed by Real-Time PCR to assess the changes in miR-155 expression.

Results: The miR-155 biomarker was positive in the peripheral blood of 25 out of 35 patients. In the group of healthy individuals, this biomarker was positive in 6 out of 35 cases. Statistical analysis of the positivity rate of this biomarker between the patient group and the healthy group showed a significant difference. Based on the findings of this study, the expression of miR-155 is increased in COPD patients compared to healthy individuals.

Conclusion: The miR-155 biomarker could potentially play an important role in the identification and diagnosis of COPD in patients. Given the growing evidence that COPD-related systemic and oral inflammation adversely affects periodontal status, jawbone health, and perioperative risk in oral and maxillofacial surgery, circulating miR-155 may also serve as a useful adjunct biomarker for risk stratification and multidisciplinary management of COPD patients in craniomaxillofacial settings. However, further studies are recommended.

Keywords: COPD; miRNA; Real-Time PCR; miR-155.

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Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a common inflammatory disease of the respiratory tract that affects the airways, lung parenchyma, and blood vessels, and is characterized by irreversible airflow limitation. COPD includes two main conditions: emphysema and chronic bronchitis. It is caused by an abnormal inflammatory response in the lungs following exposure to harmful particles or gases, which leads to airway obstruction and emphysematous changes [1]. The symptoms of COPD usually include chronic cough, excessive mucus production, air trapping, severe swelling, and shortness of breath during physical activity [2]. In fact, COPD presents symptoms beyond the lungs, with systemic manifestations such as inflammation, and is often associated with other diseases like cardiovascular conditions and metabolic syndrome [3]. Exacerbations of COPD are mainly common in patients with advanced COPD and are also linked with viral or bacterial infections [4].

In addition to inflammatory and structural cells in the lungs and pro-inflammatory mediators involved in the pathogenesis of COPD, microRNAs (miRNAs) have recently been identified as factors contributing to COPD development. miRNAs are small non-coding RNAs made up of 19-25 nucleotides [5-7]. Dysregulation of miRNA expression has been associated with human diseases, including cancer and inflammation. miR-155 is thought to play a role in lung diseases, including COPD. It is defined as a component of the early response to various inflammatory mediators in different cell types and plays a role in the differentiation of T cells into Th1 and Th2 cells [8]. Beyond the respiratory system, COPD is increasingly recognized as a systemic inflammatory disease with important implications for oral and maxillofacial health. Several epidemiological and clinical studies have reported that patients with COPD exhibit worse periodontal status, deeper periodontal pockets, greater clinical attachment loss, more gingival inflammation, and fewer remaining teeth compared with non-COPD controls [9]. chronic periodontitis and COPD are parallel neutrophil-mediated inflammatory disorders, in which dysregulated neutrophil activation, excessive protease release, and oxidative stress drive progressive destruction of connective tissue and supporting structures in both the lungs and the periodontium [10]. Identifying circulating inflammatory biomarkers such as miR-155 in COPD patients could therefore not only improve understanding of disease mechanisms but also help to stratify risk and guide multidisciplinary management in craniomaxillofacial practice. In this study, the expression of miR-155 in the peripheral blood of COPD patients was compared with healthy individuals using the Real-Time PCR technique.

Materials and Methods

Collection of peripheral blood for microRNA evaluation

This research follows a descriptive cross-sectional design where patient consent was obtained prior to sampling collection process. A total of 35 blood samples from individuals with COPD and 35 samples from healthy individuals were analyzed in this study, with participants matched by age groups. Each participant had 2 milliliters of blood drawn for research purposes, which was promptly processed for RNA extraction [11,12]. To be eligible, the study participants needed to exhibit issues like a continuous dry cough, fever, wheezing, difficulty in breathing, sputum and etc., while those with conditions, like asthma, allergies, and tuberculosis, were excluded from the study.

RNA extraction, cDNA synthesis for microRNA

This step was performed using Qiagen Cat no. 75144 RNeasy Mini Kit. The quality of the extracted RNA was checked using a nanodrop device. The ZIST ROYESH kit was used to make cDNA [13,14].

Real-time PCR reaction

The ZIST ROYESH kit contains the necessary materials to perform Real-time PCR, including forward and reverse primers and SYBR Master Mix Green. In order to normalize miRNA expression, U6 was used as a calibrator (housekeeping) [15-17]. The Real-time PCR reaction contained the following components: 3.5 µl of master mix, 1.5 µl of primer F and R each, and 2.5 µl of cDNA. The final volume was achieved by adding distilled water to bring the total volume to 20 µl. Temperatures and reaction times were adjusted according to the kit's instructions. After the end of each reaction, the results were interpreted based on the amplification and melting peak curves (Table 1).

Results

In this study, 35 peripheral blood samples of patients with COPD and 35 healthy blood samples were analyzed. These 2 groups were matched in terms of age variables. The groups were compared using a t-test in terms of average age and did not show any significant difference in terms of average age, so it can be concluded that the age factor does not cause problems in the

studied groups.

Expression analysis of studied biomarkers

Once the Real-Time PCR reaction results were gathered, the individuals who demonstrated positive biomarker expression results in both the sick and healthy groups were identified. In the peripheral blood of 25 out of 35 patients, the miR-155 biomarker was positive. In the healthy group, this biomarker was present in 6 out of 35 individuals. The positivity levels of this biomarker were compared statistically between the two groups of individuals, using a t-test method, which revealed a statistical variance between these two groups under study (P-value < 0.001).

Calculation of the difference in expression of biomarkers in two research groups

All samples were first measured for their Ct. The Ct ΔΔ method was utilized to quantify the variation in biomarker expression between the control and test samples. Furthermore, computational analysis indicates that the expression level of this marker in ill individuals is 2.5 times higher than in healthy individuals.

Table 1. Temperatures and reaction times of Real time -PCR.

Cycles	*Duration of cycles*	*Temperature*
1	*15 min*	*95°C*
35-40	*15-30 seconds*	*95°C*
	60 seconds	*55-60 °C*
1	*Melting Analysis*	*55-95 °C*

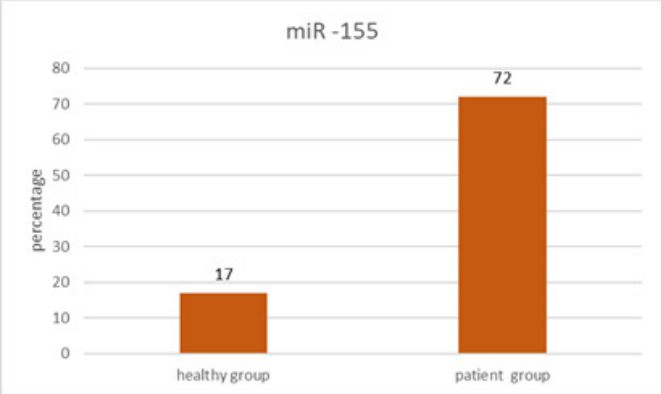

Figure 1. The percentage of miR-155 positivity in peripheral blood of patients and normal samples.

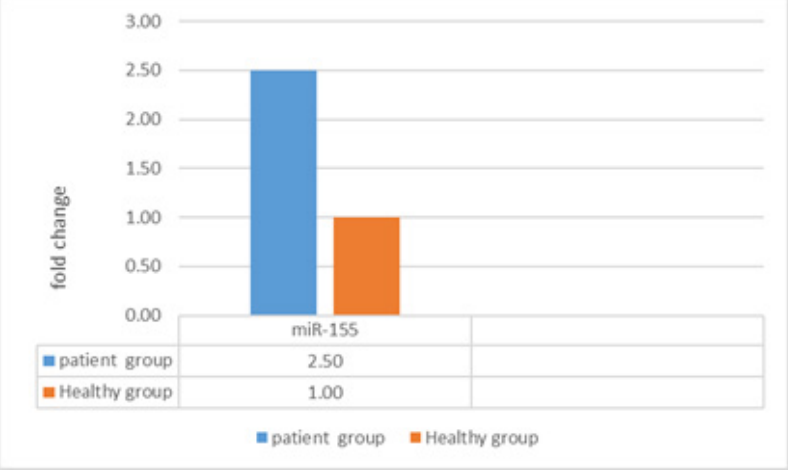

Figure 2. The difference in expression of miR-155 in the peripheral blood of patients and normal samples.

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Discussion

COPD is a chronic and heterogeneous lung disease, characterized by persistent and excessive inflammation, alveolar damage, accelerated decline in lung function, and airflow limitation [18]. Numerous factors, such as inflammation and structural cell changes, can contribute to the onset and progression of COPD. Additionally, miRNAs are involved in the pathophysiology of COPD [19]. According to Keshavarzi et al., these compounds are known to be epigenetic regulators that work by focusing on different cellular and molecular pathways [20-23]. Dysregulation of miRNAs has been shown to be has been linked to the onset and advancement of illnesses such as cardiovascular diseases, strokes, diabetes, cancer, and COPD [24]. Multiple studies have demonstrated that the upregulation or downregulation of various miRNAs (e.g., miR-21, miR-145, miR-181, miR-155, miR-144, and miR-101) may be involved in the pathogenesis of COPD [8,26].

In this study, after collecting Real-Time PCR results, individuals who tested positive for the biomarker expression in both the healthy and patient groups were identified. Among the 35 patients studied, 25 exhibited miR-155 biomarker presence in their blood, while only 6 out of 35 healthy subjects had detectable levels. Utilizing a T-test for evaluation revealed a significant contrast in biomarker expression between the patient and healthy groups. Based on a review of available literature, no previous studies have examined the prevalence of miRNAs, including the miR-155 biomarker, among healthy and diseased individuals to assess their significance. Most research has focused on investigating the role of miRNAs in the pathogenesis of COPD. Kara and colleagues evaluated the expression levels of several miRNAs, including miR-16, miR-17, miR-29c, miR-92, miR-125, miR-126, miR-146, miR-155, miR-181, and miR-122, using Real-Time PCR in 60 COPD patients [8]. Their results showed that these miRNAs were upregulated in COPD patients compared to healthy individuals. These findings suggest that these miRNAs could potentially serve as diagnostic biomarkers for COPD patients [8]. Kusko and colleagues also evaluated several genes and pathways involved in idiopathic pulmonary fibrosis (IPF) and COPD [27]. They showed that various elements of the signaling pathway are involved in the etiology of IPF and COPD. Furthermore, they demonstrated that, in contrast to the control group, IPF or COPD patients exhibit alternative splicing of molecules connected to signaling pathways. These results suggest a role for miRNA networks in regulating different signaling pathways in the pathophysiology of COPD. Further research in this area is necessary, but miRNAs may be useful as candidates for the diagnosis and prognosis of COPD.

Conflict of Interest

There is no conflict of interest to declare.

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