Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2025;50(4):1044-1052
DOI: 10.17826/cumj.1709716

RESEARCH

Investigation of MIR16-1 and MIR22 in chronic obstructive pulmonary disease

Kronik obstrüktif akciğer hastalığında MIR16-1 ve MIR22'nin araştırılması

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Abstract

Purpose: Chronic obstructive pulmonary disease (COPD), characterized by respiratory tract inflammation, is a lung disease with increasing frequency with age and high morbidity and mortality. Small non-coding RNAs with a length of 19–25 nucleotides, known as microRNAs (miRNAs), control posttranscriptional levels of gene expression. Many dysregulated miRNAs have been demonstrated to be involved in various biological pathways like inflammation, cancer, airway epithelial differentiation, and lung development. Therefore, the aim of this study was to identify if MIR16-1 and MIR22 plasma expression levels might be utilized as a criterion for the diagnosis and monitoring of COPD.

Materials and Methods: This study contained a total of 58 male individuals, 33 COPD patients and 25 healthy controls. Real-time polymerase chain reaction (RT-PCR) was employed to examine the expression levels of MIR16-1-3p and MIR22-3p. Log2−delta delta CT ($2^{-\Delta\Delta Ct}$) analysis was used to calculate miRNA expression data.

Results: No significant differences were discovered in the expression levels of MIR16-1-3p and MIR22-3p between healthy controls and COPD patients. When COPD patients were grouped as stable COPD and acute exacerbations of COPD, there was no change in the expression levels of MIR16-1-3p and MIR22-3p between different groups. Additionally, no correlation was found between the expression levels of either miRNA and the clinical features of COPD patients.

Conclusion: It was observed that there was no relationship between plasma MIR16-1-3p and MIR22-3p expression levels and COPD in this study. A deeper understanding of the connection between MIR16-1, MIR22, and COPD will be possible if the study is repeated with a wider sampling size.

Keywords: Chronic obstructive pulmonary disease, MIR16-1, MIR22, RT-PCR

Öz

Amaç: Solunum yolu iltihabı ile karakterize kronik obstrüktif akciğer hastalığı (KOAH), yaşla birlikte sıklığı artan ve yüksek morbidite ve mortaliteye sahip bir akciğer hastalığıdır. MikroRNA (miRNA) olarak bilinen 19-25 nükleotid uzunluğundaki küçük kodlamayan RNA'lar, gen ekspresyonunun transkripsiyon sonrası seviyelerini kontrol eder. Birçok düzensiz miRNA'nın inflamasyon, kanser, hava yolu epitel farklılaşması ve akciğer gelişimi gibi çeşitli biyolojik yollarda yer aldığı gösterilmiştir. Bu nedenle, bu çalışmanın amacı, MIR16-1 ve MIR22'nin plazma ekspresyon seviyelerinin, KOAH tanısı ve takibi için bir kriter olarak kullanılıp kullanılamayacağını belirlemektir.

Gereç ve Yöntem: Bu çalışma toplam 58 erkek bireyi, 33 KOAH hastasını ve 25 sağlıklı kontrolü içermektedir. MIR16-1-3p ve MIR22-3p ekspresyon seviyelerini incelemek için gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) kullanıldı. Log2−delta delta CT ($2^{-\Delta\Delta Ct}$) analizi, miRNA ekspresyon verilerini hesaplamak için kullanıldı.

Bulgular: MIR16-1-3p ve MIR22-3p ekspresyon seviyelerinde sağlıklı kontroller ve KOAH hastaları arasında anlamlı bir fark bulunmadı. KOAH hastaları stabil KOAH ve KOAH'ın akut alevlenmeleri olarak gruplandırıldığında, farklı gruplar arasında MIR16-1-3p ve MIR22-3p ekspresyon seviyelerinde bir değişiklik olmadı. Ek olarak, her iki miRNA'nın ekspresyon seviyeleri ile KOAH hastalarının klinik özellikleri arasında bir korelasyon bulunmadı.

Sonuç: Bu çalışmada, plazma MIR16-1-3p, MIR22-3p ekspresyon seviyeleri ile KOAH arasında bir ilişki olmadığı görüldü. Çalışma daha geniş bir örneklem büyüklüğüyle tekrarlanırsa, MIR16-1, MIR22 ve KOAH arasındaki bağlantının daha iyi anlaşılması mümkün olacaktır.

Anahtar kelimeler: Kronik obstrüktif akciğer hastalığı, MIR16-1, MIR22, RT-PCR

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Received: 30.05.2025 Accepted: 24.11.2025

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), the frequency of which increases with age, is a lung disease having a high rate of morbidity and death[1,2]. The disease is characterized by ongoing expiratory airflow limitation and a persistent inflammatory response[3]. Chronic exposure to harmful exogenous substances, such as tobacco, can cause alveolar and/or airway dysfunction, which impairs airflow and results in COPD. The standard of living for patients with stable COPD is reduced by lung function losses, including respiratory muscle fatigue, airflow obstruction, and airway function reduction. Hypoxemia and exacerbated hypercapnia are usually present during acute exacerbations of COPD, which are characterized by severe dyspnea and higher quantities of phlegm and phlegm purulence[4,5]. It is believed that a person's lifelong gene-environment interaction causes COPD. The gene-environment interaction is thought to cause lung damage and/or change the normal aging and development processes[6].

Small non-coding RNAs with a length of 19–25 nucleotides, known as microRNAs (miRNAs), control posttranscriptional levels of gene expression[7]. Many dysregulated miRNAs have been reported to participate in various biological pathways, such as inflammation, cancer, airway epithelial differentiation, and lung development[8]. miRNAs have the ability to reduce disease risk by acting as both a therapeutic target and a biomarker due to their endogenous nature. It's interesting to note that some circulating miRNAs, such as miR-218-5p/miR-15a and miR-122-5p, have been linked to COPD regardless of a patient's smoking history whereas other miRNAs, like Let-7, were linked to smoking[9].

MIR16-1 is a 22-nucleotide miRNA and is located in intron 6 of the deleted in lymphocytic leukemia 2 (DLEU2) gene, which is localized on chromosome 13 together with MIR15A[10]. Overexpression of MIR15A or MIR16-1 in megakaryocytic leukemia cells has been shown to induce apoptosis[11]. An earlier study indicated that acute exacerbations of COPD patients have overexpressed MIR15A and MIR16[12]. MIR15A and MIR16 have been reported to inhibit the Wnt signaling pathway, which is a hopeful target for modulating the progress of COPD[13,14]. Therefore, MIR16 may be important in stopping the development of acute exacerbations of COPD. HeLa cells are the primary source of the 22-nucleotide miRNA MIR22. Exon 2 of the MIR22 host gene (MIR22HG) contains this exonic miRNA[15]. Numerous studies have reported that MIR22 has a vital role in different pathophysiological processes, including cell division, metabolism, growth, hematopoiesis, adhesion, inflammation, tissue remodeling, apoptosis, angiogenesis, fibrogenesis, tissue homeostasis, and senescence[16,17]. Earlier researches have shown that MIR22 actively participates in the immune cascade of organisms and that dysregulation of this miRNA is linked to a variety of inflammatory conditions, such as asthma and emphysema[18,19].

Circulating miRNAs are possible disease indicators since they are resistant to endogenous RNAses and circulate in the blood[20,21]. For a number of disorders, miRNAs are now being sought after as biomarkers and potential therapeutic options. This study set out in order to determine if the expression levels of MIR16-1 and MIR22 in plasma may be utilized as a diagnostic factor in the detection and monitoring of COPD.

MATERIALS AND METHODS

Sample and procedure

Fifty-eight male individuals, 33 of whom were COPD patients (13 stable COPD, 20 acute exacerbations of COPD) and 25 healthy controls, who consulted in the Chest Diseases Polyclinic of Medical Faculty of Tokat Gaziosmanpasa University, were included in this study.

COPD was diagnosed based on the existence of symptoms, risk factors, and spirometry that demonstrates ongoing airflow restriction. In order to identify COPD, post-bronchodilator testing should demonstrate that the ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) is lower than 70%[22]. Acute exacerbations are times when symptoms of COPD go worse than they usually do on a daily basis. These episodes can linger for weeks, and severe ones can lead to hospitalization and a higher risk of mortality.

In contrast to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), which firstly focused on airflow restriction for the measurement of COPD seriousness (GOLD grades 1–4 – mild-to-very-severe COPD) to characterize patients, the ABCD assessment tool incorporates symptoms of patient (based on the COPD assessment test [CAT]

and/or modified Medical Research Council dyspnea scale [mMRC]) and exacerbation history within the last 12 months into the evaluation[22].

Future exacerbations and symptom burden are low for patients in Group A; future exacerbations and symptom burden are low for patients in Group B; future exacerbations and symptom burden are significant for patients in Group C; and future exacerbations and disease burden are high for patients in Group D.

The following are requirements for study participation: 1. Consent to participate in the study, 2. Being older than 18, 3. Having a diagnosis of COPD for the patient group, no lung or chronic disease diagnosis for the control group. Exclusion criteria for the study: 1. Having a history of asthma or severe heart failure, 2. Having a recent (within 3 months) acute coronary syndrome, pulmonary embolism or major surgery, 3. Having active cancer, 4. Being under 18 years of age.

The Ethics Committee for Non-Interventional Scientific Research of Tokat Gaziosmanpasa University, Faculty of Medicine, approved this study with the registration number 25-MOBAEK-117 in its meeting dated 08.04.2025.

Preparation of study material and real time-polymerase chain reaction (RT-PCR)

EDTA tubes containing five milliliters of blood samples underwent a 10-minute, 3000 rpm centrifugation. After centrifugation, the plasma was moved to microcentrifuge tubes and kept at -80°C for storage. These plasma samples were subjected to miRNA extraction using the SanPrep Column microRNA Mini-Preps Kit (Biobasic), according to the manufacturer. cDNA conversion was performed for the two miRNAs using the Single miRNA qPCR Assay kit, which includes cDNA synthesis mix (Atlas Biotechnologies, Türkiye). MIR16-1 and MIR22 expression levels in the plasma were next ascertained by setting up a quantitative polymerase chain reaction (qPCR) utilizing the acquired cDNAs and miR-qPCR MasterMix. The following crucial elements are included in the miR-cDNA Synthesis Kit and miR-qPCR MasterMix: antibody-mediated hot-start Taq DNA polymerase, reverse transcriptase, miRNA-specific stem-loop primer, forward and reverse primers, RNase inhibitor, $MgCl_2$, dNTPs, ROX reference dye, SBYR green dye, enhancer, and stabilizer. These components are necessary for executing reverse transcription. For RT-PCR, Applied Biosystems' 7500 Fast Real-Time PCR System (Foster City, CA, USA) was utilized. We employed the comparative threshold cycle (Ct) approach to assess the relative expression of each miRNA. Ct values of the reference gene (Rnu-6, Atlas Biotechnologies, Türkiye), which was employed for normalization, and the target genes were used in the calculations. miRNA expression data were counted using the fold change ($2^{-\Delta\Delta Ct}$) technique[23].

Statistical analysis

The statistical evaluation was determined using Version 20.0 of the IBM SPSS Statistical Package Program. The participants' attributes were reported as either mean ± standard deviation (SD) or as a number, depending on availability. Plasma miRNA expression's statistical significance was assessed using the one-way ANOVA technique. To compare COPD groups and controls pairwise, Tukey and one-way ANOVA testing were employed. Every p-value was two-tailed, and a p-value lower than 0.05 was regarded as statistically significant.

RESULTS

The average age of the 25 healthy controls was 68.16±4.930, whereas the average age of the 33 COPD patients was 67.61±6.764. Ages of COPD patients and control males were compatible with each other ($p=0.731$) (Table 1). Of 33 COPD patients, 13 (39.4%) had stable COPD and 20 (60.6%) had acute exacerbation of COPD. Table 1 displayed the clinical and demographic information for both COPD patients and controls. Age, family history, platelet (PLT), white blood cell (WBC), lymphocyte (LYM), and neutrophil (NEU) data of patients and controls were concordant with each other ($p>0.05$) (Table 1). However, the control and patient groups differed significantly in their smoking history and C-reactive protein (CRP) data ($p<0.05$) (Table 1). FEV1, FVC, FEV1/FVC, GOLD grades, GOLD groups, mMRC Dyspnea Scale stages, and oxygen saturation (SpO_2) of COPD patients were also presented in Table 1.

Table 1. Clinical and demographical characteristics of Chronic obstructive pulmonary disease patients and controls.

Characteristics	COPD patients (n=33)	Controls (n=25)	P value
Age, years	67.61±6.764	68.16±4.930	0.731
Family history, n (%)			0.725
Yes	6 (24.0)	4 (16.7)	
No	19 (76.0)	20 (83.3)	
Smoking history, n (%)			0.038
Never	0	5 (21.7)	
Former	21 (77.8)	14 (60.9)	
Current	6 (22.2)	4 (17.4)	
CRP, mg/L	53.24±88.237	14.59±33.913	0.052
WBC, per mm^3	9637.2±2459.38	8980.8±5198.28	0.521
NEU, per mm^3	7056.2±2309.92	6010.4±4344.58	0.250
LYM, per mm^3	1655.3±707.95	2023.7±951.11	0.102
PLT, per mm^3	272625.0±103926.7	231000.0±85116.8	0.116
Type of COPD, n (%)			
Stable COPD	13 (39.4)	-	
Acute exacerbations of COPD	20 (60.6)	-	
GOLD grades, n (%)			-
GOLD1	2 (8.3)	-	
GOLD2	8 (33.3)	-	
GOLD3	11 (45.8)	-	
GOLD4	3 (12.5)	-	
GOLD groups, n (%)			-
Group A	12 (41.4)	-	
Group B	0	-	
Group C	0	-	
Group D	17 (58.6)	-	
mMRC dyspnea scale, n (%)			-
Stage 0	6 (20.7)	-	
Stage 1	5 (17.2)	-	
Stage2	2 (6.9)	-	
Stage 3	11 (37.9)	-	
Stage 4	5 (17.2)	-	
FEV1, %	51.71±21.719	-	-
FVC, %	64.29±23.272	-	-
FEV1/FVC, %	62.12±13.604	-	-
SpO_2, %	92.43±4.296	-	-

Mean±standard deviation values are presented for age CRP, WBC, NEU, LYM, PLT, FEV1, FVC, FEV1/FVC and SpO2. COPD, Chronic obstructive pulmonary disease; CRP, C-reactive protein; GOLD, The Global Initiative for Chronic Obstructive Lung Disease; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; LYM, lymphocyte; mMRC, Medical Research Council (mMRC) dyspnea scale; NEU, neutrophil; PLT, platelet; SpO_2, oxygen saturation; WBC, white blood cell.

Table 2. Expression levels of MIR16-1-3p and MIR22-3p in COPD patients and controls

miRNAs	Group	N	Expression Level ($2^{-\Delta\Delta Ct}$)	p value
MIR16-1-3p	COPD patients	32	19.13±43.561	0.829
	Controls	24	16.18±58.584	
MIR22-3p	COPD patients	33	2.24±2.456	0.144
	Controls	25	4.58±8.663	

COPD, Chronic obstructive pulmonary disease.

The expression levels of MIR16-1-3p and MIR22-3p in COPD patients and healthy controls did not differ significantly from each other (Table 2) (Figure 1) (p>0.05). When COPD patients were grouped as stable COPD and acute exacerbation of COPD, MIR16-1-3p and MIR22-3p expression levels did not differ across the various groups (Table 3) (Figure 2) (p>0.05). There was also no change in the expression levels of MIR16-1-3p and MIR22-3p according to the clinical and demographic features of COPD patients (p>0.05) (data not shown).

Table 3. Expression levels of MIR16-1-3p and MIR22-3p gene in Stable COPD, Acute exacerbations of COPD and Control groups

miRNA	Group	N	Expression Level ($2^{-\Delta\Delta Ct}$)	p value
MIR16-1-3p	Stable COPD	12	22.11±58.093	0.946
	Acute exacerbations of COPD	20	17.34±33.663	
	Control group	24	16.18±50.070	
MIR22-3p	Stable COPD patients	13	2.33±3.208	0.347
	Acute exacerbations of COPD	20	2.18±1.910	
	Control group	25	4.58±8.663	

COPD, Chronic obstructive pulmonary disease.

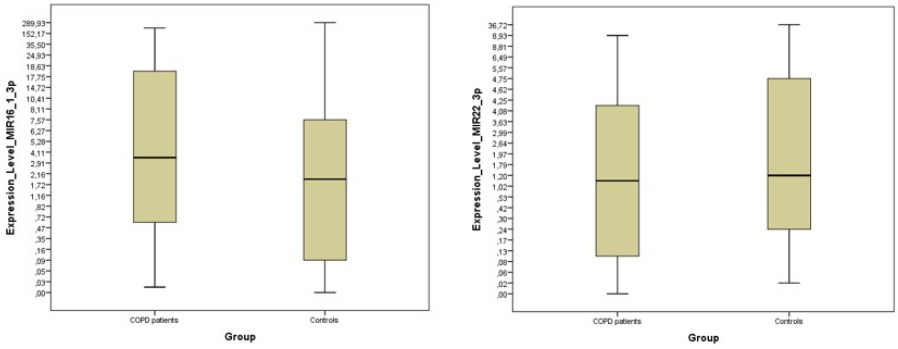


Figure 1. Comparision of expression levels of MIR16-1-3p, MIR22-3p in plasma samples of COPD patients and controls.

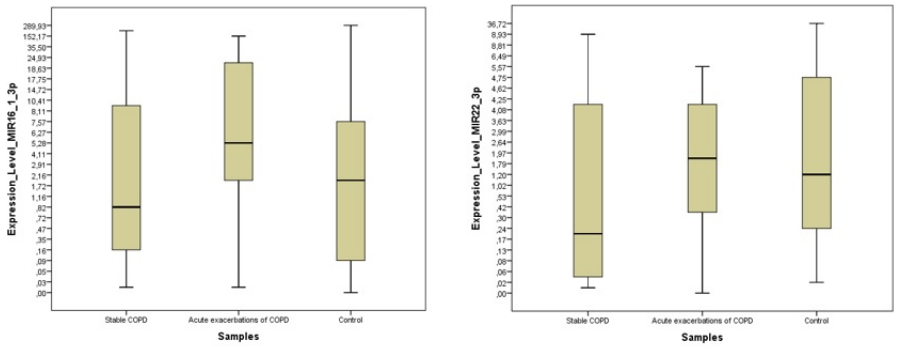


Figure 2. Comparision of expression levels of MIR16-1-3p, MIR22-3p in plasma samples of stable COPD, acute exacerbations of COPD and controls.

DISCUSSION

In the current study, we observed no significant differences in the plasma levels of MIR16-1-3p and MIR22-3p between COPD patients and healthy controls. MIR16-1-3p and MIR22-3p expression levels did not vary across the groups of COPD patients classified as having stable COPD and having an acute exacerbation of COPD, either.

miRNAs are a type of short endogenous RNA that are around 22 nucleotides long. Functionally, miRNAs interact with a target mRNA and modify its protein expression to control responses to different stimuli, cellular homeostasis, and developmental processes[24,25]. These miRNAs, which can be found in cell-free forms in various body fluids, including plasma and extracellular spaces, have the potential to constitute a category of molecular markers[26,27]. The majority of dysregulated miRNAs have been linked to cancer, inflammation, lung development, and differences in the airway epithelium[25,28,29]. It has been documented that oxidative stress and a number of miRNAs interact in pulmonary systems[30]. The upregulation of miR-34a and miR-199a-5p was found in a study where the lungs of COPD patients and histologically healthy controls were compared[31]. Some other miRNAs, such as miR-218-5p/miR-15a and miR-122-5p, have also been linked to COPD regardless of a patient's smoking history byinteracting with TGF-pathway, whereas other miRNAs, like Let-7, were linked to smoking[9].

Overexpression of MIR16-1 or its neighbor MIR15A in megakaryocytic leukemia cells has been shown to induce apoptosis via regulating antiapoptotic B cell lymphoma 2 (Bcl2) protein at the posttranscriptional level[11]. These microRNAs suppress Bcl2, which causes apoptosis in a model of leukemic cell lines[11]. This showed that MIR15A and MIR16-1 may be employed to treat malignancies that overexpress Bcl2.

An earlier study indicated that acute exacerbations of COPD patients have high expression levels of some miRNAs, such as MIR15A and MIR16, according to the microarray dataset of vastus lateralis muscle[12]. The reduction of muscular power in individuals experiencing an acute exacerbation of COPD may be linked to miRNAs (e.g., MIR1, MIR9, MIR23a, MIR15A, and MIR16), which may also serve as therapeutic targets in the treatment of acute exacerbation of COPD.

It has been demonstrated that MIR15A and MIR16 inhibit the wingless (Wnt) signaling pathway, making it a viable target for influencing the onset of COPD[13,14,32]. COPD is linked to structural alterations in the lungs and airways as well as inflammation. According to recent research, Wnt pathways play a crucial role in controlling inflammatory reactions. Wnt pathways are implicated in the development of emphysema in COPD in addition to structural alterations in the airways[13,14].

A recent review study found that a number of miRNAs, including miR16, were differently expressed in smokers and are possible biomarkers for determining a person's vulnerability to COPD[33]. In a study in which 103 COPD patients and 25 healthy controls were included and muscle-specific miRNAs were examined, although some miRNAs were associated with COPD, no association was found between the blood levels of MIR16 and COPD, consistent with our findings[34].

MIR22 has a critical role in several pathophysiological processes, including inflammation, hematopoiesis, fibrogenesis, cell division, senescence, proliferation, adhesion, apoptosis, tissue homeostasis, angiogenesis, and tissue remodeling, according to numerous studies[16,17]. MIR22 has been demonstrated to play effective roles in the immune cascade of organisms, and the dysregulation of MIR22 was associated with various inflammatory diseases, including emphysema and asthma[18,19]. In vitro studies have shown that MIR22 activates AP-1 transcription factor complexes and histone deacetylase 4 (HDAC4) to regulate antigen-presenting cell (APC) activation and TH17 responses. As a result, many human disorders that are brought on by chronic inflammation may benefit from the general therapeutic inhibition of MIR22 activity.

An earlier study has shown that serum MIR22-3p levels were downregulated in COPD caused by inhaling biomass smoke compared to COPD linked to tobacco smoking [35]. The behavior of the miR-22-HDAC4-DLCO axis was found to be varied between these two COPD groups. In our study, due to the small sample size, we were unable to differentiate COPD patients based on smoking status. This may have led to some associations being overlooked in our study. There are also other studies which have showed MIR22-5p was dysregulated in lung cancer patients compared to healthy smokers[36].

The expression of miRNAs is mostly tissue-specific; nevertheless, in addition to their intracellular effects, miRNAs can exit the cell and exert roles in other cells. So they are present in several bodily fluids: blood (serum, plasma), urine, saliva, and cerebrospinal fluid. A recent study showed that miRNA expression differences in plasma and tissue occur for some miRNAs but not for others[37]. Further studies are needed to determine these differences. However, it is also a fact that because they are easy to measure, circulating miRNAs are useful indicators of systemic illnesses and possible targets for therapy.

The most important limiting factor of this study was the small number of samples studied. A total of 58 samples were studied in this study. In miRNA studies, particularly in heterogeneous diseases such as COPD, larger sample sizes are crucial for detecting small but biologically significant changes. Therefore, the small sample size in this study may have led to the failure to detect associations identified in previous studies. In addition, the reason why there was no difference in the expression levels of both miRNAs between the stable COPD and acute exacerbation of COPD groups, may be because of the limited statistical power due to the small sample size. Also, assessing miRNA expression levels alone is insufficient to understand the complexity of COPD pathophysiology. The other limitation is the differences between control and patient groups in terms of smoking history. According to reports, smokers and non-smokers have different miRNA profiles. On the other hand, changes to the miRNA profile seem to be reversible. miRNAs regulate target genes and the proteins encoded by these genes; therefore, interpretation of the results without considering these interactions is limited. Further studies could further enhance the significance of these findings by corroborating target gene expression or protein levels in relevant pathways.

In conclusion, in this study, no association was found between plasma MIR16-1-3p and MIR22-3p expression levels and COPD. Epigenetics, such as mRNA silencing with miRNAs, has become a multidisciplinary field with tremendous scientific and therapeutic potential in the past ten years. Cancer, heart disease, behavioral issues, inflammation, aging, neurodegenerative illnesses, and diabetes have all been linked to epigenetic modifications[38]. Knowing the intricacy of the epigenetic markers linked to COPD may help identify important targets for treatment.

Author Contributions: Concept/Design : NK, SY; Data acquisition: NK, SY; Data analysis and interpretation: NK, SY; Drafting manuscript: NK; Critical revision of manuscript: NK, SY; Final approval and accountability: NK, SY; Technical or material support: NK, SY; Supervision: NK, SY; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained from The Ethics Committee for Non-Interventional Scientific Research of Tokat Gaziosmanpasa University, Faculty of Medicine, with the registration number 25-MOBAEK-117 in its meeting dated 08.04.2025.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support
Informed Consent: Informed consent was obtained from all individual participant included in the study.

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