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ORIGINAL ARTICLE

Clinical Significance of miRNA-145 and -126 in Chronic Obstructive Pulmonary Disease with Pulmonary Embolism

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SUMMARY

Background: **Pulmonary embolism (PE) a consequence of hypercoagulability status associated with chronic obstructive pulmonary disease (COPD) and worsens its course. Recently, microRNAs (miRNAs) have been linked to PE in COPD settings. We aimed to measure expression levels of miRNAs145 and 126 in COPD patients with and without PE.**
Methods: **Herein, miRNA (145 and 126) expression levels were measured in 250 COPD patients with PE by quantitative real-time PCR, and their data were compared with 300 COPD patients without PE.**
Results: **Our results showed that miRNA-145 expression was downregulated in COPD patients with PE compared to those without PE. The reverse was observed in miRNA-126 expression that was higher in COPD patients with PE than in those without PE. miRNA-145 correlated positively with FEV1/FVC and correlated negatively with D-dimer in all patients regardless of the presence of PE. In addition, miRNA-126 positively correlated with D-dimer and negatively correlated with FEV1/FVC in all studied COPD patients.**
Conclusions: **Lower levels of miRNA-145 and higher levels of miRNA-126 associated with worse diagnosis PE in patients with COPD. Extensive studies are mandated to bring a better understanding of the role of these miRNAs in the mechanism of thrombosis in COPD patients.**
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KEY WORDS

COPD, PE, miRNA-145, miRNA-126

INTRODUCTION

Chronic obstructive pulmonary diseases (COPD) are a group of abnormal conditions that affect the lung and denote the leading cause of morbidity and or mortality all over the world [1].
COPD is associated with a state of hypercoagulability and endothelial damage in more than 50% of cases that could lead to the arousal of vascular problems such as

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pulmonary embolism (PE), ischemic conditions or secondary pulmonary hypertension [2,3].
Preceding studies described an increased prevalence of PE in COPD in a series of 197 consecutive patients with COPD and reported that the frequency of PE was 25% [4], while another study recorded a lower prevalence of PE (16%) [5,6]. D-dimer is a fibrin degradation product that helps diagnose thrombosis and is associated with the extent of PE on CTPA (Computerized Tomography Pulmonary Angiography) [7].
Pathophysiological pathways clarifying how thrombosis is linked to COPD have been greatly expanded with the application of genomic studies. One developing and essential process controlling gene expression is epigenetics, which regulates gene packaging and autonomous appearance of variations in the DNA structure. Epigenetics, which involves DNA methylation, histone modifications, and activity of microRNAs (miRNAs) is providing new ways connecting genomics and environmental aspects [8].
miRNAs are gaining superior importance in research as novel organizers of gene expression and playing a central role in numerous pathophysiological pathways. It was shown that these forms of non-coding regulatory RNAs are enrolled in numerous phases of inflammation which manifest some lung illnesses such as bronchial asthma and COPD [8,9]. A number of miRNAs such as miRNA-155, miRNA-126, miRNA-21, and miRNA-146 have been described to regulate the pathogenic stimulus in the beginning and the advancement of vascular inflammatory process, neointimal injury development, atherosclerotic changes, and coronary artery disorders [9].
Preceding reports identified that miRNA-145 plays a role in adjusting the role of the airway smooth muscle (ASM), which adds to the restoration of the airway in the occurrence of COPD and its concomitant chronic inflammation. Meanwhile, Sahu and his co-authors recognized that miRNA-145 values were reduced in venous thrombosis patients and inversely correlated with amplified tissue factor (TF) levels in their cases [10]. miRNA-126 is expressed mainly in endothelial cells and has been revealed to adjust vascular uprightness and progressing angiogenesis [11].
Herein, we examined the levels of miRNA (145 and 126) expressions in COPD patients with and without PE.

MATERIALS AND METHODS

A group of 550 COPD patients was categorized based on the occurrence of PE: a group of COPD with PE (250 patients), and a group of COPD without PE (300 patients), were admitted to chest sections of two tertiary hospitals in the period from May 2016 to July 2019. Both general and chest examination were performed on all patients, COPD diagnosis was established based on the GOLD standard [12].

A written informed agreement was acquired from all patients. The study protocol was permitted by the Faculty of Medicine Ethical Committee. Rejection criteria were as follows: patients with coronary heart disorders, bronchial asthma, allergic rhinitis, and atopic conditions and patients with suspicious nodule or diagnosed as lung cancer or any current or history of previous malignancy were excluded. Sampling was done before any anticoagulation treatment had been started and patients on regular anticoagulants were excluded from the study.
All patients underwent pulmonary function evaluation of the forced expiratory volume in one second (FEV1), forced vital capacity (FVC). PEF and FEV1/FVC was carried out on all patients with respect to the global strategies [13] by means of Cosmed SrL (Quark PFTs ergo, P/N Co9035 12-99; Italy).

Blood samples

A total of 4 mL of venous sample were obtained from all participating patients, and then allocated into two tubes, ethylene diamine tetraacetic acid (EDTA) tube and sodium citrate tube.
Two milliliters of blood were added into sodium citrate (nine parts blood to one part sodium citrate), and samples were handled within 1 hour. After centrifugation at 2,000 rpm for 15 minutes at 20°C, plasma was aliquoted and kept at -80°C. All samples were liquefied for the Liatest D-Dimer test (Stago) (Diagnostica Stago, 3 allée, Asnieres sur Steine, France). Patients who recorded abnormally raised D-dimer underwent further investigation by CT pulmonary angiography to confirm pulmonary embolism. CTPA was done after sampling using a Siemens Somatom Sensation 64 CT scanner (Siemens Healthineers, Erlangen, Germany). Two milliliters blood were added to the EDTA coated tube. The tube was then centrifuged at 2,000 rpm for 5 minutes, and then plasma was transferred into an RNase-free tube for isolation of RNA. Plasma was kept at -80°C until miRNA-145 and -126 analyses were accomplished for all patients.

RNA isolation and reverse transcription (RT)

Total RNA was isolated from plasma by using RNA MiniPrep Kit (Zymoresearch, Catalog No. R2053, CA, USA). The RNA pureness and amount were determined by a Biotech Nanodrop system. Poly (A) polymerase linked enzyme (NEB, USA; Cat. no. M0276L) was used to magnify the poly-A tail of the noncoding miRNAs, and RT was done using a Thermo Scientific Reverse kit cDNA (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription and determination of miRNA expression was done by quantitative real-time PCR (qRT-PCR). The total RNA was reverse transcribed by a TaqMan MicroRNA RT kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The detected miRNAs were estimated by qRT-PCR with Taqman miRNA assay (Thermo Fisher Scientific) according to the manufacturer's instructions. miRNA-145 (assay ID 002278) and miRNA-126 (assay ID 002658).

QRT-PCR analysis of miRNAs

Quantitative real-time PCR (qRT-PCR) was done using a 7500 Fast Real-Time PCR System (Applied Biosystems, CA, USA) as follows: hot-start phase at 95°C for 7 minutes, followed by a preliminary denaturation phase for 20 seconds at 95ºC, then the annealing phase for 60 seconds at 59ºC, for 40 cycles, and lastly the extension phase for 60 seconds at 59ºC, for 40 repeated cycles. We used U6-snRNA as an internal control to normalize the CT values of the target mRNAs. The total amount of tested miRNAs was computed using the equation $2^{-\Delta\Delta Ct}$, where ΔCt = Ct (targeted miRNA) - Ct (internal control gene). All of the assays were performed in triplicate and one no-template control (NTC) was carried out in each experiment [14].

The current study was approved by the Official Review Board of Assiut and South Valley Universities, Egypt. Patients contributed to the research after explaining the objectives and steps of the study to them and attaining their agreement of sharing. Written informed consent was obtained from all enrolled patients and all the processes of the study were in accordance with the last update of the declaration of Helsinki [15].

Statistical analysis

Statistical Package for Social Sciences (SPSS), version (21) (IBM SPSS Statistics for Windows; IBM Corporation, Armonk, NY, USA) was used for data analysis. Quantitative data was presented as mean ± standard error (SEM) and qualitative data was presented in the form of frequency and percentage. Independent sample *t*-test or chi-squared test was used to determine the statistically significant variances among the study groups. Pearson's correlation was used to compare the studied parameters. $p \leq 0.05$ reflected significance.

RESULTS

COPD patients' characteristics

Table 1 showed the features of study groups of the current study. COPD patients were classified regarding the presence or absence of pulmonary artery thrombosis into a group of COPD with pulmonary embolism (250 patients) and COPD without pulmonary embolism (300 patients). The age is equivalent within the two groups with a mean ± SD of 62.1 ± 12.1 and 59.3 ± 11.1 ($p = 0.06$) for the group with PE or without PE, respectively. Concerning gender distribution, the female percentage was 35.2% and 30.7% ($p = 0.399$) for the groups with PE and without PE, respectively, with no statistical alteration. Examination of D-dimer revealed significantly higher levels in COPD patients with pulmonary embolism than those lacking embolism (mean ± SD are 2,422.4 ± 433.7, and 68.1 ± 104.3 with PE and without PE, respectively, $p < 0.0001$).

Figure 1 demonstrated a significant difference in the lung functions: FVC, FEV1, PEF and FEV1/FVC between COPD patients having a pulmonary embolism and those who did not. We observed statistically significant worse pulmonary function parameters in the group of COPD with PE than those with only COPD.

Expression patterns of miRNA-145 and miRNA-126 levels in the COPD patients with regard to the presence of PE

miRNA-145 fold change level was decreased in the group of pulmonary embolism compared to those without pulmonary embolism (mean ± SD are 5.6 ± 2.2 and 1,747 ± 689.6 for groups with PE and without PE, respectively, $p < 0.0001$ (Figure 2, right). Plasma miRNA-126 fold change level was increased in patients without pulmonary embolism compared to the PE patients (mean ± SD are 3,206.1 ± 335.4, and 674.9 ± 308.1, respectively) $p < 0.0001$ (Figure 2, left).

Correlations between miRNA-126 and miRNA-145 levels and D-dimer

Correlations between miRNA-126 and miRNA-145 fold change levels in COPD subjects were shown in Figure 3. A significant negative correlation was observed between miRNA-126 and miRNA-145 in both study groups ($R2 = 0.827$ in COPD without PE and $R2 = 0.675$ in COPD with PE and $p < 0.0001$). A significant positive correlation was detected between miRNA-126 and D-dimer level in COPD with PE ($R2 = 0.955$ and $p < 0.0001$), while a significant negative correlation was observed between miRNA-145 and D-dimer level in COPD with PE ($R2 = 0.671$ and $p < 0.0001$).

Correlations between miRNA-126 levels and pulmonary function parameters

Correlations between miRNA-126 and FVC, FEV1, PEF and FEV1/FVC in COPD with PE and COPD without PE were shown in Table 2. Significant positive correlations were observed between miRNA-126 and FVC ($R2 = 0.036$ and $p = 0.002$), significant negative correlations were observed between miRNA-126 and FEV1, PEF, FEV1/FVC in COPD with PE ($R2 = 0.057$ and $p < 0.0001$, $R2 = 0.113$ and $p < 0.0001$, and $R2 = 0.923$ and $p < 0.0001$, respectively). Also, in the group of COPD lacking PE negative correlations, Table 2 shows the correlations observed between miRNA-126 and FVC, FEV1, PEF, FEV1/FVC ($R2 = 0.208$ and $p < 0.0001$, $R2 = 0.482$ and $p = 0.151$), ($R2 = 0.249$ and $p < 0.0001$, and $R2 = 0.819$ and $p < 0.0001$, respectively).

Correlations between miRNA-145 levels and pulmonary function parameters

Correlations between miRNA-145 and FVC, FEV1, PEF and FEV1/FVC in COPD with PE and COPD without PE were shown in Table 3. Significant positive correlations were observed between miRNA-145 and FVC, FEV1, PEF, FEV1/FVC in COPD without PE ($R2 = 0.112$ and $p < 0.0001$, $R2 = 0.330$ and $p < 0.0001$, $R2 = 0.175$ and $p < 0.0001$), and $R2 = 0.787$ and $p < 0.0001$, respectively). While in group of COPD with PE, positive correlations were observed between miR-

Table 1. The characteristics and demographic data of the groups in the current study. A group of COPD with pulmonary embolism (250 patients) and a group of COPD without pulmonary embolism (300 patients).

Variables	COPD with PE (250)	COPD without PE (300)	p-value
Age (mean ± SD) (years)	62.1 ± 12.1	59.3 ± 11.1	0.06
Gender (female (%))	88 (35.2%)	92 (30.7%)	0.399
D-dimer	2422.4 ± 433.7	68.1 ± 104.3	< 0.0001
FEV1	31.5 ± 10.5	56.7 ± 27.7	< 0.0001
FVC	48.6 ± 18.1	63.4 ± 27.8	< 0.0001
PEF	24 ± 9.3	36.4 ± 20.1	< 0.0001
FEV/FVC	47.4 ± 7.6	67.5 ± 10.2	< 0.0001

Table 2. Correlation between miRNA-126 expressions (fold changes) and clinicopathological features of chronic obstructive pulmonary disease (COPD) patients.

	miRNA-126 expression (fold changes)			
	COPD with PE (250)		COPD without PE (300)	
	R2	p-value	R2	p-value
FVC	0.036	0.002	0.208	< 0.0001
FEV1	0.057	< 0.0001	0.482	< 0.0001
PEF	0.113	< 0.0001	0.249	< 0.0001
FEV1/FVC	0.923	< 0.0001	0.819	< 0.0001
D-dimer ng/mL	0.955	< 0.0001	0.64	< 0.0001

FEV1 - forced expiratory volume in one second, FVC - forced vital capacity, PEF - peak expiratory flow.

Table 3. Correlation between miRNA-145 (fold changes) and clinicopathological features of chronic obstructive pulmonary disease (COPD) patients.

	miRNA-145 expression (fold changes)			
	COPD with PE (250)		COPD without PE (300)	
	R2	p-value	R2	p-value
FVC	0.033	0.002	0.112	< 0.0001
FEV1	0.064	< 0.0001	0.330	< 0.0001
PEF	0.077	< 0.0001	0.175	< 0.0001
FEV1/FVC	0.566	< 0.0001	0.787	< 0.0001
D-dimer ng/mL	0.671	< 0.0001	0.64	< 0.0001

FEV1 - forced expiratory volume in one second, FVC - forced vital capacity, PEF - peak expiratory flow.

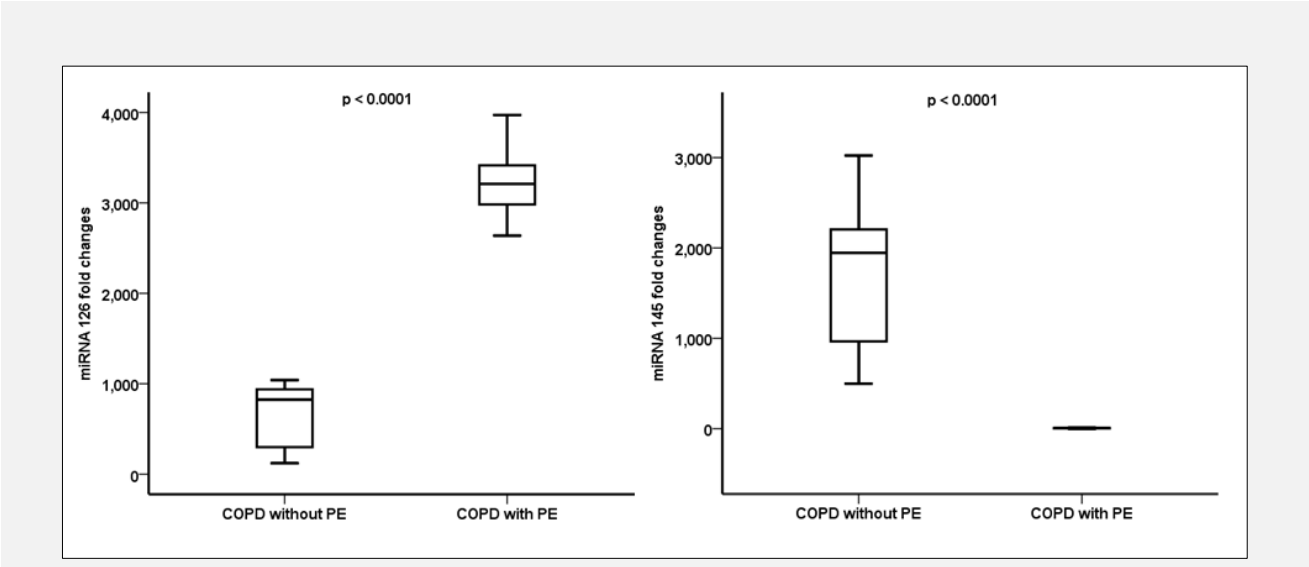

Figure 1. Difference in the lung function: FVC, FEV1, PEF and FEV1/FVC between COPD patients with and without pulmonary embolism.

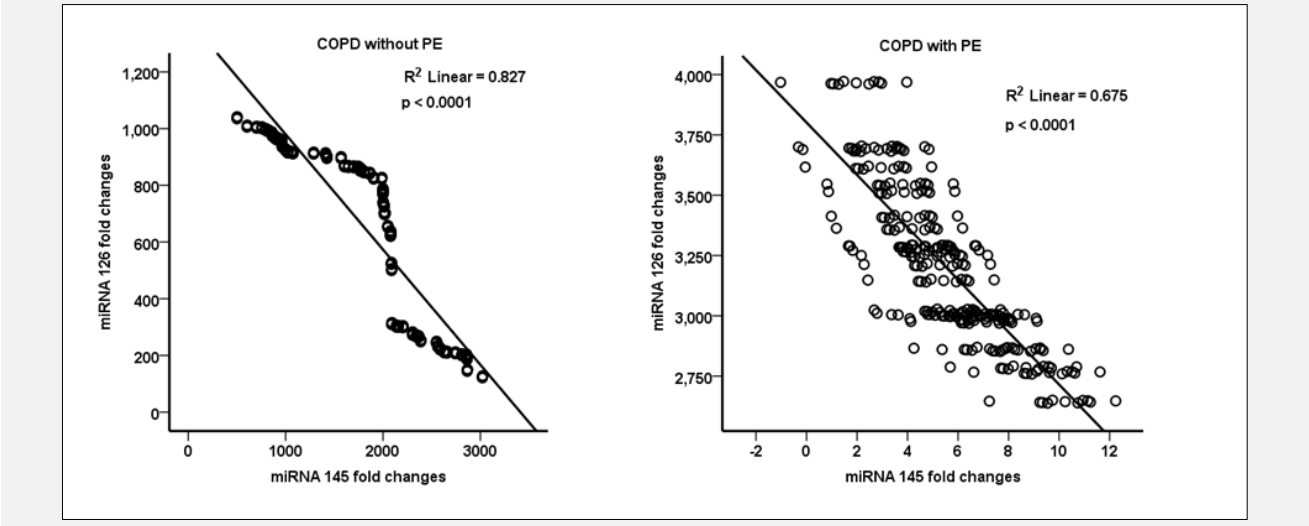

Figure 2. Expression pattern of miRNA-145 and miRNA-126 levels in the COPD patients regarding the presence of PE.

Right: miRNA-145 is significantly higher in COPD patients without PE.
Left: miRNA-126 is significantly higher in COPD patients with PE.

NA-145 and FVC, FEV1, PEF, FEV1/FVC (R2 = 0.033 and p = 0.002, R2 = 0.064 and p < 0.0001, R2 = 0.077 and p < 0.0001), and R2 = 0.566 and p < 0.0001, respectively) (Table3).

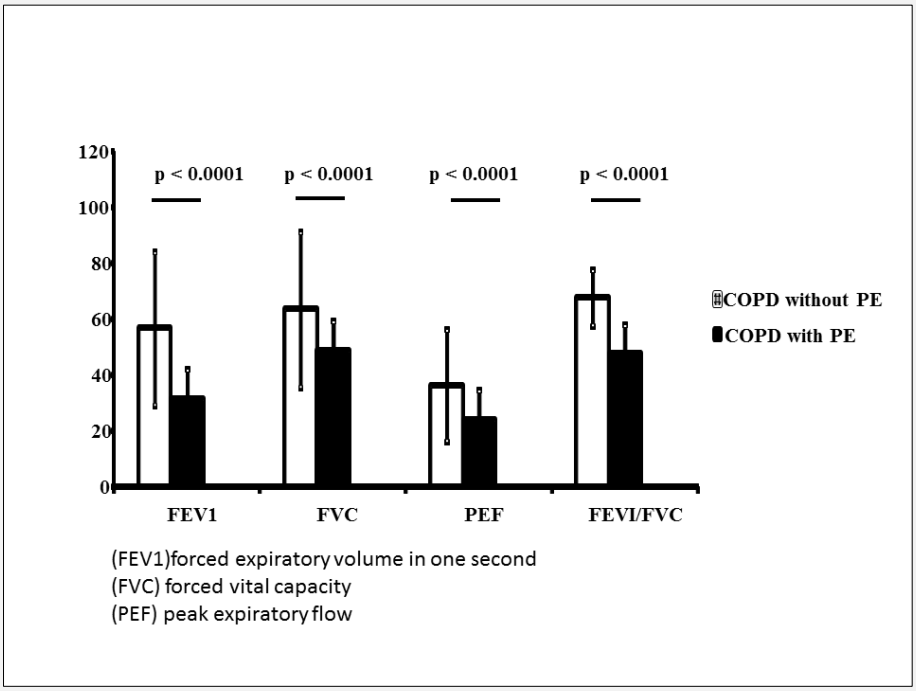

Figure 3. Correlations between miRNA-126 and miRNA-145 levels.

Right: Significant negative correlation between miRNA- 126 and miRNA-145 in COPD patients with PE.
Left: Significant negative correlation between miRNA-126 and miRNA-145 in COPD patients without PE.

DISCUSSION

Chronic obstructive lung disorders are accompanied by a high incidence of blood vessel problems as pulmonary artery thrombus formation (PE) due to the occurrence of hypercoagulability conditions in those patients. PE is a worse condition that yields noteworthy right-side heart dysfunction [3]. Latest publications point toward hemostasis disorders occurring in COPD patients and how they play a crucial role in the course of the illness and a worsened outcome. miRNA-155, miRNA-126, miRNA-21, and miRNA-145 may regulate the pathogenic signaling in the development of vascular drawbacks related to COPD [11].

In our own present cross-sectional study, we have analyzed the levels of miRNA-126 and miRNA-145 in two study groups (COPD patients, COPD problematically associated with PE). Herein, COPD patients accompanied with PE showed raised expression levels of miRNA-126 in comparison to those with no PE. In addition, patients complicated with PE revealed lower miRNA-145 expression levels when studied against those not complicated with PE.

Moreover, the fold change levels of miRNA-126 were negatively correlated with all parameters of the respiratory function test and expressed significant negative correlation with the values of D-dimer.

Interestingly, the estimated levels of miRNA-145 were positively correlated with all parameters of the respiratory function test and demonstrated a significant positive correlation with D-dimer values.

Our results were incongruent with Wang and his colleges who found higher miRNA-145 expression levels in COPD patients than controls [16] and in agreement with Shauet et al. who found reduced miRNA-145 levels in patients with venous thrombosis with an inverse trend between miRNA-145 and tissue factor expression levels [10]. This regulation may be mediated by down-regulating the tissue factor (TF) expression that is a critical trigger for the initiation of the extrinsic pathway of the coagulation cascade. miRNA-145 also promotes system cell differentiation and suppresses tumor formation by silencing gene expression networks in many cell types. It is important to note KLF4 is probably a major effector molecule for the differentiation and tumor-suppressive properties of miRNA-145. KLF4 is a validated target of miRNA-145 with a significant effect on profiles of gene expression in stem and tumor cells.

Conversely, miR-145 has been recognized by Carols and his colleagues as the furthermost talented biomark-

ers of the pathogenesis of heart and lung diseases and participates in the stabilization of atheromatous plaque [17].

Our finding that miRNA-126 expression level was higher in the COPD patients complicated with PE than those without PE was in agreement with the findings of a recent study that thrombocyte-specific over expression of miRNA-126 is concomitant with larger laser-induced thrombi than in controls, an effect that is prevented by a direct thrombin inhibitor [18]. Contrary to our findings, François Potus and his colleagues found that right-sided heart failure in patients with raised pulmonary artery pressure is associated with miRNA-126 downregulation [19].

This finding can be attributed to the fact that miRNA-126 potentiates MAP (mitogen-activated protein) kinase signaling downstream from VEGF (vascular endothelial growth factor) and FGF (fibroblast growth factor), which act as potent inducers of angiogenesis. The positive correlations between levels of miRNA-126 and thrombin generation markers in human plasma support a key role for miRNA-126 in platelet-mediated thrombin generation [18]. The endothelial cell-specific miRNA-126 signals the need for endothelial repair through its transfer from apoptotic endothelial cells in microvesicles. The pro-angiogenic consequence of miRNA-126 has been credited to the repression of SPRED-1 (Sprouty-related, EVH1 domain-containing protein 1), an adverse controller of the VEGF signaling trail, which leads to the stimulation of MAPK (mitogen-activated protein kinase) system in compliance with VEGF [18]. Our findings confirm that the environment of the lungs acts on the progression of the COPD and many biological factors participate in the pathogenesis.

CONCLUSION

miRNA-126 and -145 could be used for better understanding of the mechanism of thrombosis in COPD patients and might be used as prognostic and therapeutic targets in COPD patients to reduce the susceptibility to PE after future large scale studies.

We were restricted by the selection of only two miRNAs, while many miRNAs may be involved in COPD and PE. Thus, a large panel should be considered to know more details about their roles in COPD pathogenesis. Nevertheless, our results can encourage research in this field, even if a large-scale validation study across multiple centers is required.

Ethical Approval:

The current study was approved by the Official Review Board of Assiut and South Valley Universities, Egypt. Patients contributed to the research after explaining the objectives and steps of the study to them and attaining their agreement of sharing. Written informed consent was obtained from all enrolled patients and all the processes of the study were in accordance with the last update of the Declaration of Helsinki.

Declaration of Interest:

The authors have no conflicts of interest to announce.

References:

1. Lacedonia D, Palladino GP, Foschino-Barbaro MP, Scioscia G, Carpagnano GE. Expression profiling of miRNA-145 and miRNA-338 in serum and sputum of patients with COPD, asthma, and asthma-COPD overlap syndrome phenotype. Int J Chron Obstruct Pulmon Dis 2017;12:1811-7. (PMID: 28694694)

2. Prescott SM, Richards KL, Tikoff G, Armstrong JD, Jr., Shigeoka JW. Venous thromboembolism in decompensated chronic obstructive pulmonary disease. A prospective study. Am Rev Respir Dis 1981;123:32-6. (PMID: 7458085)

3. Gao H, Liu H, Li Y. Value of D-dimer levels for the diagnosis of pulmonary embolism: An analysis of 32 cases with computed tomography pulmonary angiography. Exp Ther Med 2018;16:1554-60. (PMID: 30112074)

4. Tillie-Leblond I, Marquette CH, Perez T, et al. Pulmonary embolism in patients with unexplained exacerbation of chronic obstructive pulmonary disease: prevalence and risk factors. Ann Intern Med 2006;144:390-6. (PMID: 16549851)

5. Rizkallah J, Man SFP, Sin DD. Prevalence of pulmonary embolism in acute exacerbations of COPD: a systematic review and metaanalysis. Chest 2009;135:786-93. (PMID: 18812453)

6. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. Eur Heart J 2014;35:3033-69, 3069a-3069k. (PMID: 25173341)

7. Ji Y, Sun B, Juggessur-Mungur KS, Li Z, Zhang Z. Correlation of D-dimer level with the radiological severity indexes of pulmonary embolism on computed tomography pulmonary angiography. Chin Med J (Engl) 2014;127:2025-9. (PMID: 24890146)

8. Perez-Sanchez C, Barbarroja N, Messineo S, et al. Gene profiling reveals specific molecular pathways in the pathogenesis of atherosclerosis and cardiovascular disease in antiphospholipid syndrome, systemic lupus erythematosus and antiphospholipid syndrome with lupus. Ann Rheum Dis 2015;74:1441-9. (PMID: 24618261)

9. Weber C, Schober A, Zernecke A. MicroRNAs in arterial remodelling, inflammation and atherosclerosis. Curr Drug Targets 2010; 11:950-6. (PMID: 20415650)

10. Sahu A, Jha PK, Prabhakar A, et al. MicroRNA-145 Impedes Thrombus Formation via Targeting Tissue Factor in Venous Thrombosis. EBioMedicine 2017;26:175-86. (PMID: 29217135)

11. Fish JE, Santoro MM, Morton SU, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 2008, 15:272-84. (PMID: 18694566)

12. Lipari M, Smith AL, Kale-Pradhan PB, Wilhelm SM. Adherence to GOLD Guidelines in the Inpatient COPD Population. J Pharm Pract 2018;31:29-33. (PMID: 29278993)

13. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med 1999;159:179-87. (PMID: 9872837)

14. Hetta HF, Zahran AM, El-Mahdy RI, et al. Assessment of Circulating miRNA-17 and miRNA-222 Expression Profiles as Non-Invasive Biomarkers in Egyptian Patients with Non-Small-Cell Lung Cancer. Asian Pac J Cancer Prev 2019;20:1927-33. (PMID: 31244320)

15. Ballantyne A, Eriksson S. Research ethics revised: The new CIOMS guidelines and the World Medical Association Declaration of Helsinki in context. Bioethics 2019;33:310-1. (PMID: 30884547)

16. Wang X, Sundquist K, Svensson PJ, et al. Association of recurrent venous thromboembolism and circulating microRNAs. Clin Epigenetics 2019;11:28. (PMID: 30760335)

17. Perez-Sanchez C, Arias-de la Rosa I, Aguirre MA, et al. Circulating microRNAs as biomarkers of disease and typification of the atherothrombotic status in antiphospholipid syndrome. Haematologica 2018;103:908-18. (PMID: 29545345)

18. Garcia A, Dunoyer-Geindre S, Zapilko V, Nolli S, Reny JL, Fontana P. Functional Validation of microRNA-126-3p as a Platelet Reactivity Regulator Using Human Haematopoietic Stem Cells. Thromb Haemost 2019;119:254-63. (PMID: 30602197)

19. Potus F, Ruffenach G, Dahou A, et al. Downregulation of MicroRNA-126 Contributes to the Failing Right Ventricle in Pulmonary Arterial Hypertension. Circulation 2015;132:932-43. (PMID: 26162916)