



Alterations in plasma miR-21, miR-590, miR-192 and miR-215 in idiopathic pulmonary fibrosis and their clinical importance

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Received: 20 May 2021 / Accepted: 2 December 2021 / Published online: 23 January 2022
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Abstract

Background Many studies have revealed that microRNA (miRNA) molecules may take part in idiopathic pulmonary fibrosis (IPF). But, the role of miRNAs in the development of IPF is not yet clear.

Methods We investigated the plasma levels of miR-21, miR-590, miR-192, and miR-215 in IPF (n = 88) and healthy control (n = 20) groups in this study. We compared the expression levels of target miRNAs in patients with IPF and healthy participants. We grouped the patients with IPF according to age, forced vital capacity, carbon monoxide diffusing capacity (DLCO), gender-Age-pulmonary physiology (GAP) score, the presence of honeycombing and compared the expression levels of target miRNAs in these clinical subgroups.

Results 82 (93.18%) of the patients with IPF were male and the mean age was 66.6 ± 8.6 years. There was no significant difference between the gender and age distributions of IPF and the control group. The mean plasma miR-21 and miR-590 levels in IPF group were significantly higher than in the control group ($p < 0.0001$, $p < 0.0001$, respectively). There was no significant difference between the miR-192 and miR-215 expression levels of the IPF and control group. Both miR-21 and miR-590 correlated positively with age ($p = 0.041$, $p = 0.007$, respectively) while miR-192 and miR-215 displayed a negative correlation with age ($p = 0.0002$, $p < 0.0001$, respectively). The levels of miR-192 and miR-215 increased as the GAP score decreased. The levels of miR-192 in patients with honeycombing were significantly lower than in those without honeycombing ($p = 0.003$).

Conclusions Our study showed that both miR-21 and miR-590 were overexpressed in IPF. The miR-21 and miR-590 were associated with DLCO, while miR-192 and miR-215 were associated with the GAP score and honeycombing.

Keywords Idiopathic pulmonary fibrosis · miR-21 · miR-590 · miR-192 · miR-215

Background

Idiopathic pulmonary fibrosis (IPF) is a progressive fatal fibrotic lung disease. Myofibroblasts related to the excessive production of the extracellular matrix appear to play a central role in the pathogenesis of this disease. But the cell types and the pathways involved in the pathogenesis have not been elucidated yet [1]. MicroRNA (miRNA) molecules are small, non-coding endogenous RNAs. They regulate the

translation of their target messenger RNAs [2]. They have a modulatory role in various cells types and almost every cellular function. Also, the majority of fibrotic processes are now thought to be regulated by miRNAs in fibrotic diseases [3]. According to recent evidence, some miRNAs which play a central role in the fibrotic signaling pathways are now also believed to be involved in the development of IPF [4]. Studies have shown that expression of miR-21 increased in the lung tissue of patients with IPF and the overexpression of miR-21 promoted lung fibroblasts [5]. In addition to its overexpression in tissue, miR-21 was also found to be high in level in the serum of patients with IPF [6]. Moreover, miR-21 levels have been associated with the diagnosis, progression, and severity of IPF recently [7].

Interestingly, another miRNA molecule, miR-590, has a similar nucleotide sequence to miR-21 [8]. It is predicted that these two miRNA molecules might share the same target

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mRNA molecules and therefore may take part in similar cellular pathways. Previously, miR-21 has been shown to contribute to both pulmonary and cardiac fibrosis processes while miR-590 has been reported to be associated with cardiac fibrosis. But, the role of miR-590 in pulmonary fibrosis has not been studied yet [9, 10].

The miR-192 molecule has also been reported to take part in the development of fibrosis. Extensive studies are being conducted on the regulatory role of miR-192 in fibrosis, particularly in renal fibrosis [11]. Furthermore, miR-192 was higher in bleomycin-induced fibrotic lungs of mice, and miR-192 was present in the sputum of patients with IPF [12, 13]. But there is no data about its relation to IPF severity. As in the case of miR-21 and miR-590, there is a strong sequence homology between the miR-192 and miR-215. Although miR-215 was reported to regulate fibroblast function, there is no study about its role in lung fibrosis [14].

Currently, the interest in the association between circulating extracellular miRNA and the development of IPF has been increasing. So, the plasma expression levels of miR-21, miR-590, miR-192, miR-215 molecules in IPF and their relationship with the clinical features were investigated in this study.

Methods

Study design and participants

This is a single-centered cross-sectional study. We conducted this study in the Chest Disease Department of Akdeniz University Hospital between June 2018 and August 2020. The IPF group consisted of IPF patients who met the criteria of the 2018 American Thoracic Society/European Respiratory Society Clinical Practice Guidelines [15]. We excluded the ones with end-stage renal disease, hepatic failure, acute coronary heart disease, or cancer in the study. The control group consisted of healthy individuals that matched the IPF group's age and gender. None of the participants in the control group displayed any symptoms or had any preexisting disease. We performed an electrocardiogram and a blood test to check heart enzymes and kidney and liver function in all participants. We excluded the ones with any abnormality in these tests.

Clinical features

We obtained the data about age, gender, smoking history, symptoms, drug therapy, lung biopsy of the IPF patients. We used recent high-resolution computer tomography (HRCT) images. We accepted subpleural and basal dominant reticulation with honeycombing as usual interstitial pneumonia (UIP). We classified as probable UIP if there

was no honeycombing and all other HRCT findings were the same as UIP. We defined those patients with very limited subpleural reticulation or ground-glass opacities without other obvious features of pulmonary fibrosis, as indeterminate UIP pattern. We also compared the miRNA levels of the IPF patients with and without honeycombing on HRCT. We used a modified Medical Research Council (mMRC) Questionnaire for the assessment of the severity of dyspnea. We calculated the gender-age-pulmonary physiology (GAP) score [16]. We performed spirometry and helium diffusion tests. We obtained blood samples of IPF patients for miRNA analysis simultaneously with these tests.

We also obtained data about the age, gender, smoking history, and comorbidities of the healthy control subjects. We collected blood samples of the participants in the control group for miRNA analysis. But, we did not perform spirometry and helium diffusion tests since all were healthy individuals.

We grouped the IPF patients according to age, FVC (% of predicted), DLCO (% of predicted), and GAP score. We grouped the patients < 65 years as a younger group and ≥ 65 years as an older group. We formed three subgroups based on FVC values (< 65%, 65–80%, > 80%), DLCO values (< 45%, 45–70%, > 70%), and GAP scores (0–2, 3–4, 5–8) [17, 18].

miRNA expression analysis

We examined the expression levels of target miRNA molecules by real-time PCR. We obtained plasma from peripheral blood samples and stored it at – 80 °C. We isolated miRNA from plasma with microRNA Purification Kit (Norgen Biotek Corp., Canada) and synthesized cDNA by using miRCURY LNA RT Kit (Qiagen, USA). We performed real-time PCR with miRCURY LNA SYBR Green PCR Kit (Qiagen, USA) for hsa-miR-21-5p, hsa-miR-590-5p, has-miR-192-5p, has-miR-215-5p, and hsa-miR-16-5p on LightCycler 480 II instrument via LightCycler 480 1.5 software (Roche Diagnostics GmbH, Germany). We used REST-MCS software and miR-16 as a reference for the relative quantification analysis of target miRNA genes [19].

Human ethics

We obtained written informed consent from all patients and control subjects. The Clinical Research Ethics Committee of Akdeniz University Faculty of Medicine approved the study on 17.01.2018 (Decision No: 53, Dated: 17.01.2018). Akdeniz University Scientific Research Projects Coordination Unit supported the study (Project Number: TTU-2019-4793).

Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism 9 (GraphPad Software, USA) program. Descriptive statistics for categorical variables were reported as number (n) and percentage (%) and for continuous variables as mean \pm standard deviation (SD) or mean \pm standard error of mean (SEM) values. Differences of means between two independent groups were examined with the Mann–Whitney U test, and between more than two independent groups with Kruskal Wallis test. Relationships of two independent categorical variables were interpreted by Chi-square analysis or with Fisher's exact Test. The correlation of independent continuous variables was analyzed with Spearman's Rank Correlation test. In all analyses, a value of $p \leq 0.05$ was considered to be statistically significant.

Results

Demographic features, respiratory functions, and radiological findings

Eighty-eight patients with IPF and 20 healthy control subjects were evaluated in this study. IPF group comprised 82 (93.18%) males and the mean age was 66.6 ± 8.6 years (Table 1). There was no significant difference in gender distribution and the mean age of the IPF and the control groups. There was more smoker in the IPF group than in the control group (71% vs. 45%, $p = 0.038$). But, there was no significant

Table 1 Demographic features, respiratory functions and radiological findings of participants

Characteristic	Control n=20	IPF n=88
Age, mean \pm SD	63 ± 7.2	66.6 ± 8.6
Gender (male)	80%	82%
Smoking history	45%	71%*
Smoking amount (P/Y), mean \pm SD	31.8 ± 30.3	31.8 ± 18.3
Dyspnea	–	81.8%
FVC (%pred.), mean \pm SD	–	71.3 ± 17.1
DLCO (%pred.) (n=82), mean \pm SD	–	60.6 ± 17.8
GAP score, median (min–max)	–	3 (0–8)
HRCT (with honeycombing) (n=84)	–	76%
Drug therapy (n=66)	–	75%

IPF Idiopathic pulmonary fibrosis, P/Y packet/year, SD standard deviation, FVC forced vital capacity, %pred. % of predicted, DLCO diffusing lung capacity for carbon monoxide, GAP gender-age-pulmonary physiology, min minimum, max maximum, HRCT high-resolution computed tomography, UIP usual interstitial pneumonia, n number

* $p = 0.038$, smoking rate in IPF versus control with Fisher's exact test

difference in smoking amount between the IPF and the control group. The majority of the patients (81.8%) had dyspnea, in various degrees and the median mMRC score was 2. The mean FVC (% of predicted) was $71.3 \pm 17.1\%$ and the mean DLCO (% of predicted) was $60.6 \pm 17.8\%$.

Sixty-four (72.7%) of the IPF patients had a UIP pattern and 24 patients (4 with indeterminate UIP and 20 with probable UIP) had a surgical biopsy for the diagnosis. The mean age of patients with honeycombing on HRCT was significantly higher than that of patients without honeycombing (68.5 vs. 62.1 years, $p = 0.0027$). The median GAP score was 3 and antifibrotic drugs were being taken by 75% of the patients. While the majority (74%) of the patients were taking pirfenidone (mean dose 2400 mg/day), the rest of them were using nintedanib (mean dose 275 mg/day).

The clinical features of the IPF patients differed between the younger and older groups, while gender and the smoking features were similar in both age groups (Supplemental Table 1). Honeycombing was significantly more common ($p = 0.015$) and the GAP score was higher ($p = 0.001$) in the older group however, the mean FVC and the mean DLCO values were similar.

miRNA levels in IPF and control group

The mean plasma miR-21 and miR-590 levels in IPF were at least two-fold higher than in control group (Fig. 1). There was no significant difference in the mean plasma miR-192 and miR-215 levels between the IPF and control groups. The plasma levels of target miRNA molecules were not altered significantly with smoking amount in both the control (miR-21: 0.65 ± 0.35 vs. 0.77 ± 0.64 , $p > 0.05$;

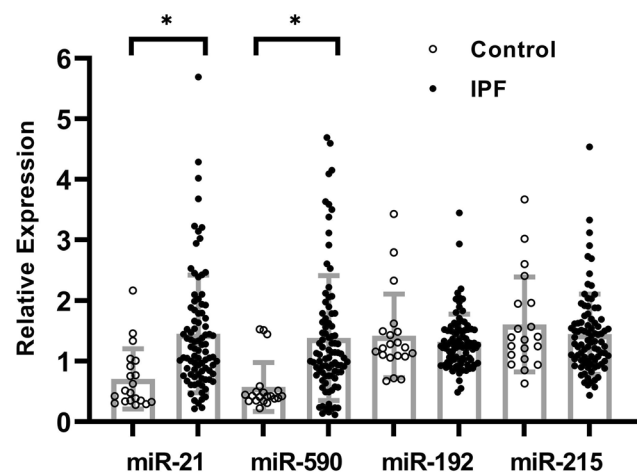


Fig. 1 Plasma expression levels of miR-21, miR-590, miR-192, and miR-215 in IPF and control groups. The relative expression level of the target miRNAs was calculated by using miR-16 as a reference. Bars represent means and error lines indicate standard deviation. * $p < 0.0001$

miR-590: 0.52 ± 0.31 vs. 0.63 ± 0.51 , $p > 0.05$; miR-192: 1.50 ± 0.85 vs. 1.31 ± 0.45 , $p > 0.05$; miR-215: 1.60 ± 0.86 vs. 1.60 ± 0.71 , $p > 0.05$; smoking history no vs. yes, respectively) and IPF groups (miR-21: 1.49 ± 1.13 vs. 1.43 ± 0.90 , $p > 0.05$; miR-590: 1.31 ± 0.90 vs. 1.41 ± 1.08 , $p > 0.05$; miR-192: 1.29 ± 0.58 vs. 1.33 ± 0.40 , $p > 0.05$; miR-215: 1.35 ± 0.57 vs. 1.48 ± 0.69 , $p > 0.05$; smoking history no vs. yes respectively). Additionally, there was no significant difference between the mean target miRNA levels of patients taking anti-fibrotic drugs and those not taking anti-fibrotic drugs (miR-21: 1.33 ± 0.78 vs. 1.48 ± 1.03 , $p > 0.05$; miR-590: 1.20 ± 0.97 vs. 1.44 ± 1.04 , $p > 0.05$; miR-192: 1.35 ± 0.55 vs. 1.31 ± 0.42 , $p > 0.05$; miR-215: 1.46 ± 0.71 vs. 1.44 ± 0.65 , $p > 0.05$; anti-fibrotic therapy no vs. yes, respectively). A positive correlation was found between the levels of miR-21 and miR-590, and between the levels of miR-192 and miR-215 in both IPF and control groups (Supplemental Fig. 1).

The effect of age on serum miRNA levels

In the IPF group, there was a positive correlation between age and both miR-21 and miR-590, while miR-192 and miR-215 were negatively correlated with age (Fig. 2). The mean miR-21 and miR-590 levels were significantly higher in the older group than in the younger group (1.1 ± 0.6 vs. 1.6 ± 1.0 , $p = 0.032$, 1.0 ± 0.5 vs. 1.6 ± 1.2 , $p = 0.039$, respectively). miR-192 and miR-215 levels were significantly lower in the older group than in the younger group (1.5 ± 0.5 vs. 1.2 ± 0.3 , $p = 0.004$, 1.6 ± 0.6 vs. 1.3 ± 0.6 , $p = 0.001$, respectively).

In the control group, none of the miRNA molecules correlated with age (Fig. 2). The control subjects were separated into younger ($n = 12$) and older ($n = 8$) age groups. The levels of miR-21 and miR-590 were similar in both subgroups. But the levels of miR-192 and miR-215 were significantly

higher in the older group than in the younger group (miR-192: 1.2 ± 0.7 vs. 1.6 ± 0.5 , $p = 0.047$, miR-215: 1.3 ± 0.7 vs. 1.9 ± 0.6 , $p = 0.031$).

Association of miRNAs with pulmonary function tests, GAP score and radiological findings in patients with IPF

Among the FVC subgroups, no difference was determined in plasma levels of miR-590, miR-192, and miR-215, but there was a slight difference in the miR-21 level (Supplemental Table 2). The plasma miR-21 level of patients with FVC $> 80\%$ was higher than that of patients with FVC 66–80% (1.6 ± 0.9 vs. 1.2 ± 0.9 , $p = 0.012$).

In DLCO subgroups, there was no significant difference in miR-192 and miR-215 levels (Supplemental Table 2). Both miR-21 and miR-590 were reduced in patients with DLCO $< 45\%$ compared to other DLCO subgroups, but the miR-21 and miR-590 levels of this group were similar to those of control subjects (Fig. 3). The plasma miR-21 and miR-590 levels were not significantly changed among DLCO subgroups (via Kruskal Wallis test). But the patients with the highest DLCO levels showed significantly increased miR-590 levels compared to patients with the lowest DLCO values (1.5 ± 0.9 vs. 1.0 ± 1.1 , $p = 0.022$).

miR-21 and miR-590 levels did not change according to the GAP score (Supplemental Table 3). In contrast, miR-192 and miR-215 were reduced along with the increased GAP scores [Fig. 3]. The plasma miR-192 level was significantly reduced in the patients whose GAP scores are higher than 2 ($p = 0.010$). Although the plasma miR-215 levels were not significantly changed with the GAP score, their levels were reduced in the patients with a GAP score higher than 2 ($p < 0.05$ with Mann Whitney U test).

According to the radiological findings, miR-21 and miR-590 levels were not different (Supplemental Table 3).

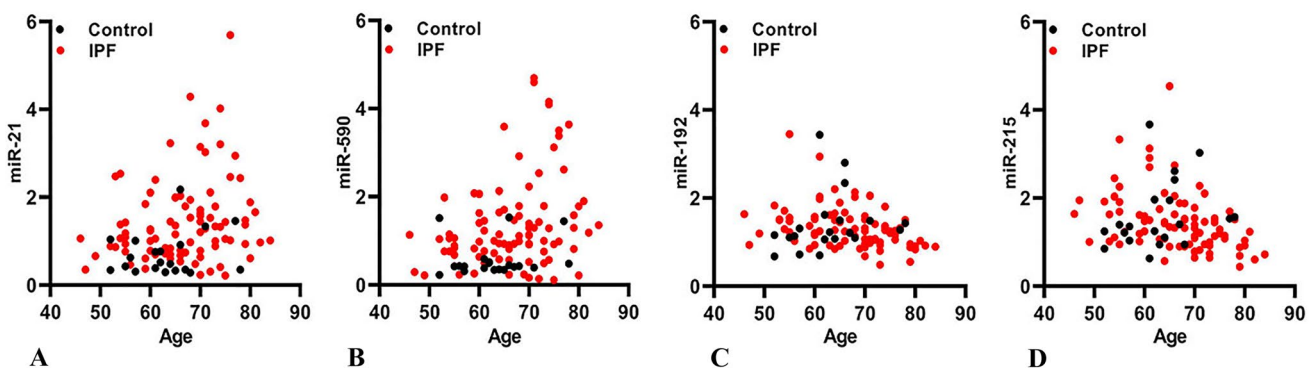


Fig. 2 Correlation of age with miRNA in IPF and control groups. **A** Correlation of age with miR-21 (control: $r = 0.06$, $p > 0.05$, IPF: $r = 0.21$, $p = 0.041$). **B** Correlation of age with miR-590 (control: $r = 0.17$, $p > 0.05$, IPF: $r = 0.28$, $p = 0.007$). **C** Correlation of age

with miR-192 (control: $r = 0.42$, $p > 0.05$, IPF: $r = -0.38$, $p = 0.0002$). **D** Correlation of age with miR-215 (control: $r = 0.42$, $p > 0.05$, IPF: $r = -0.44$, $p < 0.0001$)

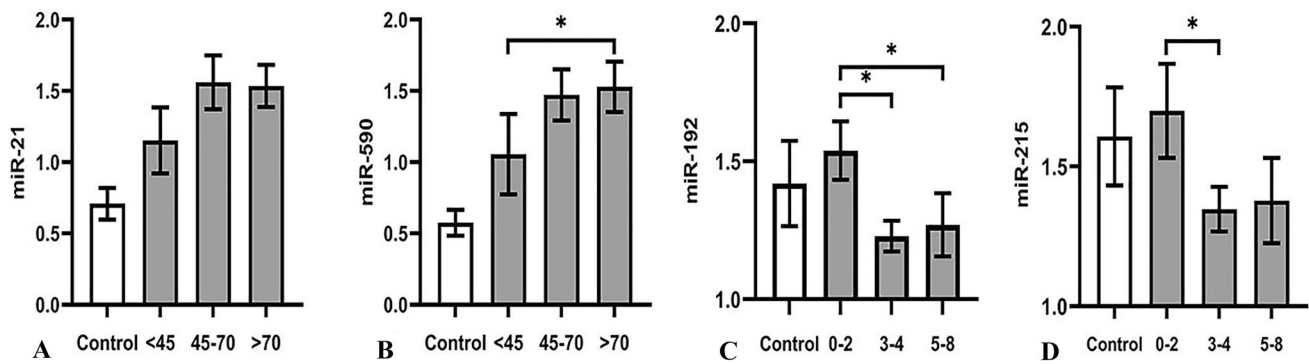


Fig. 3 miRNA levels according to the DLCO and GAP score subgroups. **A** Plasma miR-21 and **B** miR-590 levels among the subgroups of DLCO <45%, 45–70%, and >70% were not significantly different via the Kruskal–Wallis test. **C** Plasma miR-192 levels among the GAP score subgroups were significantly different via the Kruskal–Wallis test ($p=0.010$). With Dunn’s multiple comparison

test, the GAP 0–2 subgroup was significantly different from other subgroups. **D** Plasma miR-215 levels among the subgroups were not significantly different via the Kruskal–Wallis test but there was a significant difference between the miR-215 levels of GAP 0–2 and 3–4 subgroups with the Mann–Whitney U test ($p=0.035$). Bars represent means and error lines indicate SEM. * $p<0.05$

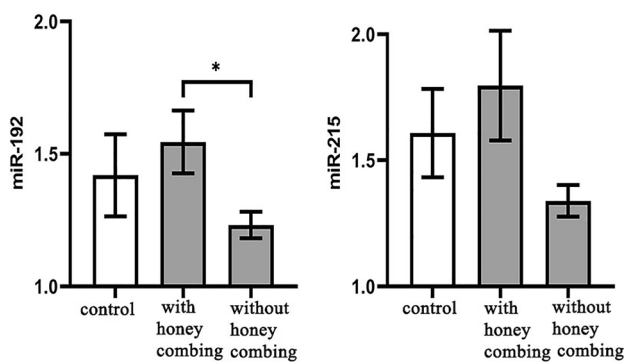


Fig. 4 miR-192 and miR-215 levels according to the presence of honeycombing on HRCT. **A** Plasma miR-192 levels of the patients with honeycombing were reduced compared to the patients without it ($p=0.003$). **B** Plasma miR-215 levels were similar between the patients with honeycombing or without honeycombing ($p>0.05$). Bars represent means and error lines indicate SEM. * $p<0.05$

The plasma miR-215 levels were lower in the patients with honeycombing compared to those without honeycombing, but the difference was not significant (Fig. 4). Among the patients with honeycombing, plasma miR-192 levels were significantly lower than in those without honeycombing ($p=0.003$).

Discussion

In this study, we investigated plasma miR-21, miR-590, miR-192, and miR-215 levels in IPF patients. The plasma miR-21 and miR-590 levels in the IPF group were significantly higher than those of the control group. But there was no significant difference between the miR-192 and miR-215

levels of the IPF and control groups. Moreover, we found an association between all target miRNAs and age. Besides that, we observed that miR-21 and miR-590 were associated with DLCO, while miR-192 and miR-215 were associated with GAP score and honeycombing. These findings suggest that miR-21 and miR-590 may play a role in the development of IPF and can be used for diagnostic purposes. Additionally, we suggest that both miR-192 and miR-215 can be utilized to assess IPF progression.

The miRNA molecules have been reported to be involved in lung fibrosis. Studies have shown that miR-21 expression is increased in the lung tissue of patients with IPF [5]. Serum miR-21 is suggested as a representative of miR-21 expression in lung tissue of IPF patients. Because miR-21 levels are increased in the serum of patients with IPF, as in the lung tissue [20, 21]. In our study, we also found that the plasma miR-21 levels were significantly higher among IPF patients than in the control group, concordant with previous studies.

To the best of our knowledge, there is no knowledge about the plasma levels of miR-590, miR-192, and miR-215 in patients with IPF. Although each of these miRNAs has been reported to play a role in various fibrotic diseases, only miR-192 has been associated with lung fibrosis so far [22, 23]. miR-192 expression was found to be both elevated in the sputum of IPF patients and to be a modulator of airway remodeling in patients with asthma [13, 14]. In this study, the plasma miR-590 levels were significantly higher in IPF than in the control. But there was no significant difference in miR-192 and miR-215 levels between the IPF and control groups.

Besides being associated with the presence of IPF, our study provided evidence that plasma miRNAs could display expressional alterations according to the severity of the IPF. The mean plasma level of miR-590 was high in the IPF

group. This finding suggests that plasma miR-590 expression increases with IPF development. But plasma miR-590 levels decreased along with decreasing DLCO. The decrease in miR-590 expression during disease progression suggests that there might be a negative feedback mechanism. Besides this, the plasma levels of miR-192 did not change in the presence of IPF. But they decreased along with the increase in the radiological severity or the GAP score. In the model of bleomycin-induced fibrosis, the miR-192 expression had also decreased as the fibrosis progressed [12]. Thus, the miR-192 could be a promising plasma biomarker for the prognosis and progression of IPF. Further investigation of changes in miR-192 expression in later stages of pulmonary fibrosis is needed.

In this study, we observed that miR-21 correlated with miR-590 in both control and IPF groups. But, miR-21 and miR-590 had before displayed an inverse pattern of expression in ulcerative colitis. [24]. So, miR-21 and miR-590 may show different expression patterns in different diseases. Moreover, miR-21 and miR-590 showed a correlation with the age of the IPF patients in our study. A previous study also revealed that circulating levels of miR-21 increase along with age, and miR-590 levels are higher in older people [25]. The recognition of miR-21 as a DNA damage-dependent circulatory biomarker for aging also supports these findings [26]. We observed that miR-192 and miR-215 also correlated with age in the IPF group. The levels of miR-21 and miR-192 were higher in the circulating vesicles of aged mice [27]. The miRNA molecules in the circulation are distributed in several compartments as protein-bound or in exosomes. Currently, we don't know whether these different compartments are necessarily associated with specific diseases. [28]. The effect of age on miRNA levels may depend on the severity of the disease and the specific compartment of miRNAs in which they are distributed.

The interaction of miRNAs with fibrotic processes could be through some signaling pathways. TGF β molecule, associated with miR-21, is an important regulator in pulmonary fibrosis. It induces miR-21 expression and upregulates miR-21 via Smad3 [29]. Moreover, miR-21 inhibits Smad7 and further stimulates TGF β signaling [30, 31]. Lung injury stimulates both miR-21 and TGF β expression and inhibits Smad7 expression [32]. All these suggest that miR-21 may be a positive feedback regulator of TGF β , the main driver of fibrosis.

Together with miR-21, miR-590 takes part in fibrotic TGF β signaling. TGF β reduces miR-590 expression. MiR-590 directly inhibits both TGF β and TGF β receptors to suppress its signaling pathway. It also inhibits the expression of Smad3 and Smad7 [33, 34]. So, miR-590 and TGF β molecules seem to act as negative regulators of each other. But, in our study, we found that the plasma levels

of miR-590 were higher in IPF patients than in healthy individuals. A possible explanation for this discrepancy might be in the inflammatory response. Pro-inflammatory cytokines regulate miR-590 expression by the JAK/STAT pathway [35]. During inflammatory processes, STAT3 which is a target of miR-590 activates STAT5, and STAT5 increases miR-590 expression [36]. It has previously been demonstrated that both STAT3 and STAT5 proteins play a role in the regulation of TGF β signaling and the Epithelial-Mesenchymal Transition in pulmonary fibrosis [37]. This suggests that miR-590 may play a dual role in both inflammation and fibrosis as a negative feedback regulator in IPF.

Previously, it was reported that the miR-192 expression was lower in cigarette smoke-exposed lung tissue [38]. Additionally, exosomal miR-192 was present in high amounts in the sputum of the patients with IPF [13]. TGF β -induced miR-192 functions seem to be much more heterogeneous. Because miR-192 can inhibit or stimulate fibrosis depending on the cell type. The TGF β promotes miR-192 expression by signaling with Smad3, while it reduces miR-192 expression via Smad7 [39]. In our study, the levels of miR-192 in IPF were not significantly different than those in healthy individuals. But, miR-192 displayed a positive correlation with GAP score and honeycombing. This suggests that we may use miR-192 to assess IPF progression.

There are some limitations in this study. The participants in the control group were small in number. Secondly, the correlation of target miRNAs with age in control group needs to be further analyzed with additional studies. Finally, we don't know whether the miRNAs in the plasma of the IPF patients originated from lung tissue or extra-pulmonary tissues, or even from circulating cells.

In conclusion, we found the plasma miR-590 and miR-21 levels were significantly higher in the IPF group than in the control group. We observed that all target miRNAs correlated with age. Moreover, the miR-21 and miR-590 levels were associated with DLCO, while miR-192 and miR-215 levels were associated with GAP score and honeycombing. These miRNAs may play a regulatory role in the development and progression of IPF. They may be used as a diagnostic or prognostic tool and even, as a new therapeutic target. Nevertheless, we need further research to better understand the roles of these plasma miRNAs in the development, progression, and severity of IPF.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11033-021-07045-x>.

Funding The study was supported by Akdeniz University Scientific Research Projects Coordination Unit (Project Number: TTU-2019-4793).

Declarations

Conflict of interest All authors confirm that all data underlying the findings are fully available without restriction and have no conflict of interest to declare.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by The Clinical Research Ethics Committee of Akdeniz University Faculty of Medicine (Decision No: 53, Dated: 17.01.2018).

Informed consent Informed consent was obtained from all individual participants included in the study.

References

- Selman M, King TE, Pardo A, American Thoracic Society, European Respiratory Society American, College of Chest Physicians (2001) Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 134(2):136–151
- He L, Hannon GJ (2004) MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 5:522–531
- Bowen T, Jenkins RH, Fraser DJ (2013) MicroRNAs, transforming growth factor beta-1, and tissue fibrosis. *J Pathol* 229(2):274–285
- Martinez FJ, Collard HR, Pardo A et al (2017) Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers* 3:17074
- Liu G, Friggeri A, Yang Y et al (2010) miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 207(8):1589–1597
- Tsitoura E, Wells AU, Karagiannis K et al (2016) MiR-185/AKT, and miR-29a/collagen 1a pathways are activated in IPF BAL cells. *Oncotarget* 7(46):74569–74581
- Makiguchi T, Yamada M, Yoshioka Y et al (2016) Serum extracellular vesicular miR-21-5p is a predictor of the prognosis in idiopathic pulmonary fibrosis. *Respir Res* 17(1):110
- Ren J, Zhang J, Xu N, Han G, Geng Q, Song J, Li S, Zhao J, Chen H (2013) Signature of circulating microRNAs as potential biomarkers in vulnerable coronary artery disease. *PLoS One* 8(12):e80738
- Climent M, Viggiani G, Chen YW, Coulis G, Castaldi A (2020) MicroRNA and ROS crosstalk in cardiac and pulmonary diseases. *Int J Mol Sci* 21(12):4370
- Yuan X, Pan J, Wen L et al (2020) MiR-590-3p regulates proliferation, migration, and collagen synthesis of cardiac fibroblast by targeting ZEB1. *J Cell Mol Med* 24(1):227–237
- Jenkins RH, Martin J, Phillips AO, Bowen T, Fraser DJ (2012) Pleiotropy of microRNA-192 in the kidney. *Biochem Soc Trans* 40(4):762–767
- Cushing L, Kuang PP, Qian J et al (2011) miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol* 45(2):287–294
- Njock MS, Guiot J, Henket MA et al (2019) Sputum exosomes: promising biomarkers for idiopathic pulmonary fibrosis. *Thorax* 74(3):309–312
- Lou L, Tian M, Chang J et al (2020) MiRNA-192-5p attenuates airway remodeling and autophagy in asthma by targeting MMP-16 and ATG7. *Biomed Pharmacother* 122:109692
- Raghu G, Remy-Jardin M, Myers JL, American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, Latin American Thoracic Society et al (2018) Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med* 198(5):e44–e68
- Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS, Poletti V, Buccioli M, Elicker BM, Jones KD, King TE Jr, Collard HR (2012) A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med* 156(10):684–691
- Kolb M, Collard HR (2014) Staging of idiopathic pulmonary fibrosis: past, present, and future. *Eur Respir Rev* 23(132):220–224
- American Medical Association (2008) Guides to the evaluation of permanent impairment, 6th edn. American Medical Association, Chicago
- Pfaffl MW, Horgan GW, Dempfle L (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* 30(9):e36
- Li P, Zhao GQ, Chen TF et al (2013) Serum miR-21 and miR-155 expression in idiopathic pulmonary fibrosis. *J Asthma* 50(9):960–964
- Li P, Li J, Chen T, Wang H, Chu H, Chang J, Zang W, Wang Y, Ma Y, Du Y, Zhao G, Zhang G (2014) Expression analysis of serum microRNAs in idiopathic pulmonary fibrosis. *Int J Mol Med* 33(6):1554–1562
- Lin X, Steinberg S, Kandasamy SK et al (2016) Common miR-590 variant rs6971711 present only in African Americans reduces miR-590 biogenesis. *PLoS One* 11(5):e0156065
- Lesizza P, Prosdocimo G, Martinelli V et al (2017) Single-dose intracardiac injection of pro-regenerative microRNAs improves cardiac function after myocardial infarction. *Circ Res* 120(8):1298–1304
- Naghdalipour M, Moradi N, Fadaei R et al (2020) Alteration of miR-21, miR-433, and miR-590 tissue expression related to the TGF- β signaling pathway in ulcerative colitis patients. *Arch Physiol Biochem* 15:1–5
- Olivieri F, Rippo MR, Monsurrò V et al (2013) MicroRNAs linking inflame-aging, cellular senescence, and cancer. *Ageing Res Rev* 12(4):1056–1068
- Olivieri F, Albertini MC, Orciani M et al (2015) DNA damage response (DDR) and senescence: shuttled inflamma-miRNAs on the stage of inflame-aging. *Oncotarget* 6(34):35509–35521
- Olivieri F, Spazzafumo L, Santini G et al (2012) Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging. *Mech Ageing Dev* 133(11–12):675–685
- Jung HJ, Suh Y (2014) Circulating miRNAs in ageing and ageing-related diseases. *J Genet Genom* 41(9):465–472
- Zhong X, Chung AC, Chen HY, Meng XM, Lan HY (2011) Smad3-mediated upregulation of miR-21 promotes renal fibrosis. *J Am Soc Nephrol* 22(9):1668–1681
- Wang JY, Gao YB, Zhang N, Zou DW, Wang P, Zhu ZY, Li JY, Zhou SN, Wang SC, Wang YY, Yang JK (2014) miR-21 overexpression enhances TGF- β 1-induced epithelial-to-mesenchymal transition by target smad7 and aggravates renal damage in diabetic nephropathy. *Mol Cell Endocrinol* 392(1–2):163–172
- Marts LT, Green DE, Mills ST et al (2017) MiR-21-mediated suppression of Smad7 induces TGF β 1 and can be inhibited by activation of Nrf2 in alcohol-treated lung fibroblasts. *Alcohol Clin Exp Res* 41(11):1875–1885
- Yu ZW, Xu YQ, Zhang XJ et al (2019) Mutual regulation between miR-21 and the TGF β /Smad signaling pathway in human bronchial fibroblasts promotes airway remodeling. *J Asthma* 56(4):341–349
- Ekhteraei-Tousi S, Mohammad-Soltani B, Sadeghizadeh M et al (2015) Inhibitory effect of hsa-miR-590-5p on cardiosphere-derived stem cells differentiation through downregulation of TGF β signaling. *J Cell Biochem* 116(1):179–191

34. Jafarzadeh M, Soltani BM (2016) Hsa-miR-590-5p interaction with SMAD3 transcript supports its regulatory effect on the TGF β signaling pathway. *Cell J* 18(1):7–12
35. Favreau AJ, Sathyanarayana P (2012) miR-590-5p, miR-219-5p, miR-15b, and miR-628-5p are commonly regulated by IL-3, GM-CSF, and G-CSF in acute myeloid leukemia. *Leuk Res* 36(3):334–341
36. Cocolakis E, Dai M, Drevet L, Ho J, Haines E, Ali S, Lebrun JJ (2008) Smad signaling antagonizes STAT5-mediated gene transcription and mammary epithelial cell differentiation. *J Biol Chem* 283(3):1293–1307
37. Dong Z, Tai W, Lei W, Wang Y, Li Z, Zhang T (2016) IL-27 inhibits the TGF- β 1-induced epithelial-mesenchymal transition in alveolar epithelial cells. *BMC Cell Biol* 1(17):7
38. Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S (2009) Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. *FASEB J* 23(3):806–812
39. Lan HY (2012) Smads as therapeutic targets for chronic kidney disease. *Kidney Res Clin Pract* 31(1):4–11

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