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MicroRNA 153 as epigenetic biomarker for diagnosis of idiopathic pulmonary fibrosis

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Abstract Unregulated microRNAs (miRNAs) are associated with the development of idiopathic pulmonary fibrosis (IPF) because they chang the expression of genes included in the fibrotic process. Therefore, This study aims to determine the relationship between the level of expression of miRNA-153 in IPF patients, assess its diagnostic accuracy, and explore its correlations with inflammatory blood parameters and lung function. So, these analyses have been evaluated as a total of 120 subjects, with 60 being the IPF and 60 being the age and gender matched healthy control subjects. Using TRIzol reagent, RNA was extracted from blood samples , with no significant differences in age (p=0.113), gender distribution (p=0.232), or BMI (p=0.808), ensuring a well-matched cohort. Using quantitative reverse transcription polymerase chain reaction (PCR) and stem-loop analysis, the gene expression of both miR-153 and GAPDH (the mutated gene) was determined. Analysis of the results confirmed the gene expression values calculated from 2^−ΔCt analysis. The gene expression of miRNA-153 was compared between IPF patients and the healthy control group, and the results showed that the average gene expression of miRNA-153 was 0.25 ± 0.06 and 2.66 ± 0.91 in IPF patients and the healthy control group, respectively; the average levels were lower in IPF patients compared to the healthy control group, and the difference was highly statistically significant ($P < 0.001$). that indicates a 90.6% decrease in expression, or approximately a 10.6-fold decrease, Analysis of the operational characteristics curve (ROC) showed that miR-153 is an excellent diagnostic biomarker for differentiating between IPF patients and healthy individuals. The significant downregulation of circulating miR-153 in IPF along with its exceptional diagnostic performance underscores its promise as a non-invasive biomarker for early detection and monitoring of the disease in idiopathic pulmonary fibrosis.

Keywords: biomarker, idiopathic pulmonary fibrosis, microRNA-153, RT-qPCR, serum expression

Introduction Idiopathic pulmonary fibrosis is an interstitial lung disease that continues to worsen over time. It is characterized by repeated respiratory epithelial injury, extreme extracellular fibroblast matrix deposition, and resultant lung scarring which causes respiratory failure. Ther is also the lack of Early diagnosis alongside insufficient treatment options that IPF requires, which has resulted in a major focus on the molecular pathways of fibrogenesis. The latest guidelines covering interstitial lung diseases and the so-called “progressive pulmonary fibrosis (PPF)” concept reinforce the need for more accurate molecular therapies and biomarkers (1, 2). MicroRNAs and other small non-coding RNAs have proven to be of great importance in lung fibrosis, as they regulate pathways that drive epithelial stress responses, fibroblast-to-myofibroblast transitions, immune-stromal crosstalk, and remodeling the fibroblast extracellular matrix. Reviews estimate that in IPF lung tissue, 10% of miRNAs with imbalanced expression are classified as pro-fibrotic or antifibrotic owing to the TGF-β/Smad, MAPK, and PI3K/AKT pathways (5). Evidence suggests that circulating miRNAs within exosome or protein complexes could also serve as non-invasive biomarkers for the presence and progression of disease (3, 4). In IPF

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research, miRNAs are the focus of special attention because of their dual roles as biomarkers and disease regulators (3, 4). miRNA-153 (miR-153) has captured interest for its purported anti-remodeling activity within pulmonary vascular models, suggesting its involvement within pathway nodes intersecting with fibroblast activation and tissue remodeling. For example, miR-153's overexpression HPASMCs under hypoxic conditions led to ROCK1 and NFATc3 downregulation and cellular proliferation and migration inhibition. In vivo studies also demonstrate that miR-153 promotes apoptosis of proliferative vascular cells, indicating a possible silencing of maladaptive growth/survival programming (7). While these studies largely pertain to pulmonary hypertension, the involvement of Rho-kinase and NFAT signaling concerning fibroblast activation and mechanotransduction offers biologic plausibility for the role of miR-153 in fibrotic lung conditions (6, 7). The currents of IPF focus on the epithelial injury and the EMT crossroads as fibrotic lungs phenomena, considering the presence of miR-153 modulation on EMT and fibroblast activity to be more than plausible. The IPF requires operational frameworks and management plans that drove the reasons to formulate guiding principles. The mechanistic studies of miRNAs in lung remodeling, and more so the molecular behavior of miR-153, offer positive cause to sustain dedicated research probing miR-153 as a plausible modulation and/or biomarker.Hence, the current study investigated the diagnostic relevance and expression profiles of free circulating miR-153 in newly diagnosed IPF and matched controls.

Materials and Methods

Study Design and Ethical Approval

This case-control study aimed to assess the difference in expression of circulating microRNA– 153 (miR-153) in patients with idiopathic pulmonary fibrosis (IPF) and age and sex matched healthy control patients. The study gained the IRB approval, and all procedures were in accordance with the Declaration of Helsinki. Each participant gave the written informed consent before they were registered in the study. 120 participants in total were included in the study which consisted of 60 patients with clinically confirmed idiopathic pulmonary fibrosis and 60 normal control subjects who were devoid of any respiratory illnesses. The patients were diagnosed with idiopathic pulmonary fibrosis (IPF) as per the latest ATS/ERS/JRS/ALAT (2022) international guidelines which includes and not limited to clinical, radiological, and physiological assessments. Every participant was given an exhaustive clinical evaluation which included taking a medical history, doing a physical examination, and assessing the medical files. The criteria of inclusion in the patient group consisted of having a restrictive lung function pattern accompanied with high resolution CT having characteristic interstitial pneumonia (UIP). While exclusion criteria were having a Chronic Obstructive Pulmonary Disease, any connective tissue pathological disorder, or any other interstitial lung disease secondary to other primary illnesses. Controls were chosen so that the patients were matched for age, gender, and body mass index (BMI) in order to reduce confounding variables. The participants' detailed demographic information, smoking status, and family history of respiratory disorders were collected through interviews and standard forms.

Sample Collection and Processing

Peripheral venous blood samples (5 mL) were taken from each participant in a sterile environment and using two different types of vacutainer tubes. The first tube which had an EDTA anticoagulant was used for a complete blood count (CBC) analysis and the second tube is referred to as Rnalater (Thermo Fisher Scientific, USA) which is used to RNA stabilizes so that the RNA is ready for downstream analysis involving miRNA extraction. The samples were placed in a cooler in ice and immediately transferred to a molecular biology lab in which they were analyzed within an hour after having been drawn. Analysis of leukocyte counts, differential counts, and neutrophil/lymphocyte ratios were performed on the processed EDTA samples using an automated hematology analyzer (Sysmex XN-Series, Sysmex Corporation, Kobe, Japan). In parallel, to the aforementioned analysis, tubes containing Rnalater were centrifuged at 3,000 rpm for 10 minutes in order to separate the plasma which was then aliquoted and stored at -80 to await isolation of the RNA. RNase-free materials and equipment were used for these procedures. Some of the demographic and clinical parameters which were age, BMI, and smoking history were used to assess miRNA expression levels.

Assessment of Lung Function

All participants underwent pulmonary function testing using a calibrated spirometer (CareFusion, Germany) according to ATS/ERS standards (2019). The tests included determining the percentage of predicted forced vital capacity (FVC, % predicted) and the ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC %), which helped ascertain the presence of IPF.In the analysis, each subject was credited with a minimum of three acceptable maneuvers which resulted in the FVC and FEV_1 pull values of greatest volume. Daily 3-L syringe spirometer calibrations helped ensure quality control. The values calculated were based on the predicted age, sex, height, and ethnicity values. In order to minimize inter-operator variability, every single technician carried out the tests. IPF patients who had disparate airflow limitation patterns were incorporated.

Hematological Evaluation

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EDTA blood samples were taken and studied to find the hematological characteristics of systemic inflammation in IPF patients. The hematology parameters were processed through the Sysmex XN-Series automated analyzer which automated the blood analysis. Total white blood cell (WBC) count, and Neutrophil and Lymphocyte counts with the Neutrophil percentage are the characteristic parameters.As with prior calibration sessions, quality control was carried out with the provided calibration standards. Neutrophil-to-lymphocyte ratio (NLR) 'proxy' marker of systemic inflammatory load. Data are presented as the mean ± standard deviation (SD) of the IPF and Control groups. Indices were subsequently correlated with expression levels of miR-153 to investigate the inflammatory response to putative genic regulation.

RNA Extraction and Quality Assessment.

Total RNA, as well as small RNA fractions, was obtained from frozen whole blood samples with The use of the Trizol reagent (Bioneer, Korea. RNA was collected according to protocol as described by the manufacturer. The obtained RNA underwent the following: "phase separation," paraffin-embedded blocks of tissue that was treated with alkaline isopropanol, then precipitated, and overly collected on the upper interface, washed with 75 percent ethanol, and finally suspended in RNase free water. Co-precipitated and then brought to a certain concentration by spectrophotometric techniques using the NanoDrop 2000 (Thermo Fisher Scientific, USA) at A260/A280 and A260/A230 ratios. Samples with a ratio of 1.8-2.1 for downstream applications were accepted.To evaluate the quality of RNA samples, electrophoresis on 1% agarose gel was conducted and the presence of intact rRNA bands was confirmed. RNA samples were stored in a -80 degree freezer until reverse transcription. To avoid contamination, negative extraction controls were used. To avoid RNA degradation, the isolated RNA was used immediately to synthesize cDNA. To enhance reproducibility and reliability of gene expression results, duplicate samples were processed. Reverse transcription was performed using the adjustable and automatic micropipette. Each sample was transferred to a clean assay strip and corresponding PCR tubes for subsequent steps. The samples were held at 4 degrees until they were loaded onto the PCR thermocycler for programmed steps. Preheating to 25 degrees was the first step, subsequently lasting 1 minutes, this was the reverse transcription step, in which the cDNA of the selected miRNA was synthesized. The reverse transcription temperature was subsequently elevated to 40 degrees for 60 minutes and finally, control samples and experimental samples were each stored in a -20 degree freezer for future use.Quantitative PCR was conducted with the Bio-Rad CFX96 real-time system (USA) with SYBR Green Master Mix (Promega, USA). In each 20 µL reaction, the final concentrations of reagents were 2 µL of the cDNA template, 10 µL of SYBR Green master mix, 0.5 µL of each of the forward and reverse primers, and the balance was made up with nuclease-free water. The cycling conditions used were 95 °C for 2 min, followed by a further 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Melt curve analysis was done with the aim of confirming specificity. Each run was made to exclude non-template controls in order to assess contamination. All reactions were done in duplicates to maintain the repetition and make sure there is no differential bias in the data.

Primer Design

GAPDH and hsa-miR-153 primers were designed through NCBI and Primer3-Plus web applications and were synthesized by Macrogen Co. (Korea). The reverse transcription universal primer sequence was GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCA CTGGATACGACTTTTTTTTTTTVN. Forward primer for miR-153 is AAGCGACCTTATAATACAACCTGAT, while for GAPDH, the primers are AAAATCAAGTGGGGCGATGC and TTCTCCATGGTGGTGAAGACG for the forward and reverse primers, respectively.

Primer specificity was checked by BLAST and the standard curve method was used to verify amplification efficacy. All primers produced individual peaks during melt curve analysis which suggests strong specificity. The PCR efficiencies were within range (95 % and 103 %) which is acceptable for relative quantification studies.

Statistical Analysis and Quantification

Statistical analysis was done on the 2^–ΔCt method results which used GAPDH for normalization. Fold changes were established by comparison to one calibrator sample belonging to the control group. The analyses were done on data constructed using SPSS version 26 (IBM Corp., USA) and Microsoft Excel 2010. The Kolmogorov–Smirnov test was used to check data for normality and results are presented in mean ± SD. Independent-samples t-tests or Chi-square calculations were used to establish group comparisons between patients with IPF and control participants.

Area under the curve (AUC) analysis evaluated the diagnostic effectiveness of miR-153 through receiver operating characteristic (ROC) curve analysis, reporting sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) along with the AUC. In this regard, a p-value of less than 0.05 was significant. The relationship of miR-153 expression and the various clinical or laboratory (WBC, FVC %, FEV_1/FVC %) values were determined through correlation analysis with the use of Pearson correlation in an attempt to identify relevant possible functional relationships.

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Results

Demographic Characteristics of the Study Population

This study had a total of 120 participants, of which 60 were diagnosed with idiopathic pulmonary fibrosis (IPF) and 60 were healthy individuals matched for age, gender, and body mass index (BMI) as controls. Patients with IPF had a mean age of 58.23 ± 11.9 years, while the control group had a mean age of 53.95 ± 11.6 years. This difference was not statistically significant (p = 0.113). The age group frequency distribution also showed no significant association (p = 0.651). IPF subjects comprised 65 % males, while the control subjects comprised 75 % males, which suggested a similar gender composition (p = 0.232). The two groups had a similar demographic composition which minimized the confounding impact of non-disease variables such as age and gender, meaning the differences in the expression of miR-153 were more likely to be attributed to disease rather than demographic factors.

IPF patients had an average body mass index (BMI) of 23.1 ± 3.96 kg/m² while the controls had an average BMI of 22.9 ± 3.69 kg/m² (p = 0.808). The majority of participants in both groups were classed as normal weight (70 % in IPF vs. 73.3 % in controls), with low levels of obesity. These data points affirm the demographic and anthropometric similarity of the groups under study, supporting the balanced foundation for the molecular work to come. (Table 1)

Table 1: Demographic data of patients with idiopathic pulmonary fibrosis (IPF) and controls.

	IPF Patients *n* = 60	**Healthy control *n* = 60**	***P***
Age (years)			
Mean ±SD	**58.23 ± 11.9**	**53.95 ± 11.6**	0.113 † NS
Range	36–85 years	30– 77 years	
< 40 years, *n* (%)	9 (15.0)	12 (20.0%)	0.651 ¥ NS
40-49 years, *n* (%)	12 (20.0%)	15 (25.0%)	
50-59 years, *n* (%)	12 (20.0%)	8 (13.3%)	
≥ 60 years, *n* (%)	27 (45.0%)	25 (41.7%)	
P value	0.004**	0.015**	
Gender			
Male, *n* (%)	39 (65.0 %)	45 (75.0 %)	0.232 ¥ NS
Female, *n* (%)	21 (35.0%)	15 (25.0%)	
M:F ratio	1.86:1	3:1	
P value	0.020**	0.001**	
Smoking			
Smoking, *n* (%)	36 (60.0%)	2 (3.3%)	0.001 ¥ S
Non-smoking, *n* (%)	24 (40.0%)	58 (96.7%)	
Body mass index (BMI) kg/m2			
Mean ±SD	**23.1 ± 3.96**	**22.90 ± 3.69**	0.808 † NS
Range	18.70–31.80	18.90– 30.80	
Obesity Classification			
Normal weight, *n* (%)	42 (70.0%)	44 (73.3%)	0.893 ¥ NS
Overweight, *n* (%)	14 (23.3%)	13 (21.7%)	
Obese, *n* (%)	4 (6.7%)	3 (5.0%)	

Family History Section

A review of family histories showed that 9 (15%) of IPF patients had a positive family history of pulmonary fibrosis, and 51 (85%) had no such family history. None of the control subjects had a family history of fibrotic lung disease. The low number of familial cases suggests that, although a genetically determined risk is possible, most of the patients with IPF in the study population had the disease due to non-hereditary or environmental factors. Comparing miR-153 levels between the two groups showed no significant (NS) difference. p-value (0.841).The pattern of distribution is consistent with other

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studies showing that familial IPF is uncommon and often associated with mutations of the telomerase or surfactant pathways. The current study did not find significant differences in clinical or molecular features between patients with and without a positive family history, suggesting that the lack of tension on a family tree (the 'miR-153 downregulation' family) is not the result of some common ancestor (Figure 1).

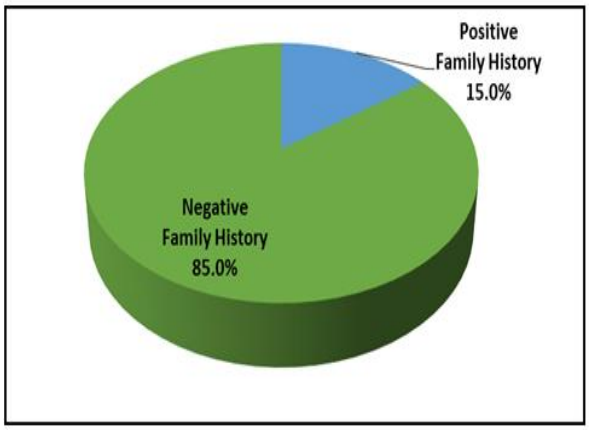

Figure 1: Family history of patients with idiopathic pulmonary fibrosis.

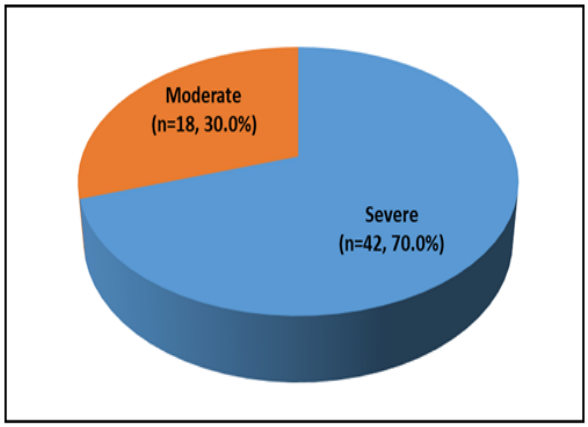

Figure 2: The Frequency distribution of patients suffering from idiopathic pulmonary fibrosis (IPF) with respect to the severity of the disease.

ICD 10 disease severity IPF

Regarding the severity of disease, the majority of patients with IPF (42 patients; 70%) were classified with severe disease, while 18 patients (30%) were classified as moderate. This evaluation was predicated on lung function measures and the FVC % and diffusing capacity reduction observed as clinical surrogates for the fibrotic burden. The predominance of severe cases is in line with the usual clinical presentation pattern at referral centers, where advanced disease is the target of attention. Subsequent analyses sought to study the alteration of the molecular profile of disease progression to determine whether mature miR-153 expression correlates with the progression of the disease. There is no significant difference in miR-153 levels between moderate and severe cases p-value = 0.104, indicating the direction of the relationship (NS).

Lung Function Parameters

The results of the pulmonary function tests corroborated the presence of the restrictive ventilatory defect characteristic of idiopathic pulmonary fibrosis (IPF). The mean FVC % in patients was 41.81 ± 6.11, significantly lower than 97.00 ± 4.36 recorded for healthy controls ($p < 0.001$). In contrast, the FEV_1/FVC % ratio was higher in patients (91.64 ± 10.1) than controls (80.45 ± 1.41, $p < 0.001$), which is indicative of restrictive physiology. An inverse relationship was demonstrated by logistic regression analysis between FVC % and FEV_1/FVC %, signifying that lower FVC % is associated with higher FEV_1/FVC % due to the presence of conserved airflow.Analyses revealed no clinically important differences in FVC % or FEV_1/FVC % between patients with and without a family history ($p > 0.05$), but both worsened significantly with greater disease severity ($p = 0.001$). These results affirm the previous hypothesis that the decline in pulmonary function is associated with the progressive formation of fibrosis in IPF. Furthermore, the results position FVC % as a clinically valuable measure to correlate to molecular expression patterns (Table 2).

Table 2: Comparison of idiopathic pulmonary fibrosis patients and healthy controls in lung function parameters (FVC % and FEV_1/FVC %).

Groups		Forced Vital Capacity (FVC%) level	FEV1/FVC%1
IPF patients	Mean ± SD	41.81 ± 6.11	91.64 ± 10.1
	Range	23.99-59.02	63.19-100.0
Control	Mean ± SD	97.00 ± 4.36	80.45 ± 1.41
	Range	86.38-105.26	77.78-83.44
p-value		0.001**	0.017**

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Hematological Findings

The results of the hematological investigations highlighted the presence of systemic inflammation in the patients with IPF. The mean white blood cell count (WBC) was 8.89 ± 1.8 × 10⁹/L and 7.82 ± 1.3 × 10⁹/L for the controls ($p = 0.015$), so the difference in samples taken was statistically significant. Similarly, in IPF absolute neutrophil counts and the percentages of neutrophils were increased (6.70 ± 1.2 × 10⁹/L and 75.11 ± 10.1 % respectively) as compared to controls (5.23 ± 1.1 × 10⁹/L and 63.81 ± 4.46 %, $p < 0.05$). These increases indicate the presence of a chronic inflammatory response in the body that leads to continuous tissue damage and scarring. In contrast to this, IPF patients had lower counts of lymphocytes (1.66 ± 0.41 × 10⁹/L) in comparison to the controls (2.19 ± 0.32 × 10⁹/L, $p = 0.001$). Family history and duration of the disease did not have considerable influence over the hematological parameters, but the severity of the disease had a positive correlation with % of neutrophils and a negative correlation with the count of lymphocytes. The increased neutrophil-to-lymphocyte ratio substantiates the idea of chronic inflammation being a persistent underlying condition for the fibrotic changes in the tissue remodeling (Table 3).

Table 3: Comparison of hematological parameters (WBC, neutrophils, lymphocytes, and the % of neutrophils) in patients with idiopathic pulmonary fibrosis and the healthy control group.

Characteristic	IPF patients n=60	Healthy control n=60	P
White blood cells (WBC) count			
Mean± SD	8.89 ± 1.8	7.82 ± 1.3	0.015 † S
Range	5.24 – 13.86	5.25 – 11.06	
Neutrophils count			
Mean± SD	6.70 ± 1.2	5.23 ± 1.1	0.001 † S
Range	3.64–11.70	3.44 – 7.79	
Lymphocyte count			
Mean± SD	1.66 ± 0.41	2.19 ± 0.32	0.001 † S
Range	0.45 – 2.65	1.08 – 2.60	
Neutrophils%			
Mean± SD	75.11 ± 10.1	63.81 ± 4.46	0.002 † S
Range	60.50 – 94.60	59.10 –70.40	

Gene Expression of miR-153

In patients with IPF as compared to the control, a distinct downregulation of gene expression of miR-153 was observed in the quantitative real-time PCR analysis. The miR-153 amplification mean Ct value in the patient group was 28.63 while in the controls it was 25.34 suggesting the diseased subjects had a lower level of abundance of this miRNA. The expression values after normalization to GAPDH were 0.25 ± 0.06 for patients and 2.66 ± 0.91 for controls ($p < 0.001$) which indicates a 10-fold decrease of miR-153 expression in the patients with IPF. The fold-change analysis showed the level of expression of miR-153 in IPF blood samples was 250% lower than the expression level in a healthy person. The Receiver Operating Characteristic (ROC) analysis also supported the diagnostic value of miR-153 expression and resulted in AUC = 1.000 with 100% sensitivity and specificity at a cutoff value <0.68. This further establishes miR-153 as a robust non-invasive biomarker for differentiating IPF patients. Correlation analysis revealed significant negative correlation between miR-153 expression and FVC %($r = –0.613$, $p = 0.001$) and also with the neutrophil percentage ($r = 0.341$, $p = 0.008$) suggesting the lower the expression of miR-153, the more the pulmonary restriction and systemic inflammation (Figure 3).

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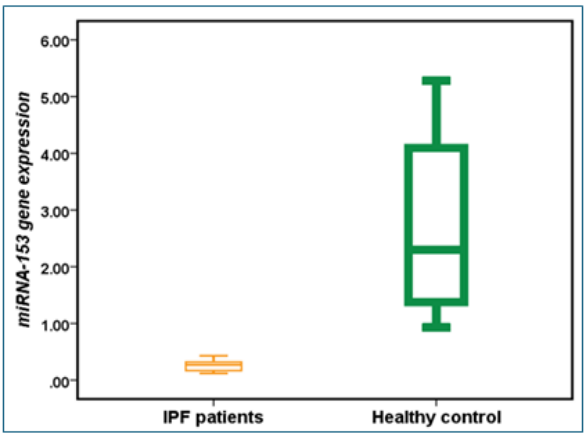

Figure 3: The diagnostic value and relative expression levels of circulating miR-153 in IPF patients as compared to healthy controls.

Correlation of miR-153 Expression with Clinical and Hematological Parameters

Additional analysis was performed to investigate whether the expression of miR-153 is linked to any particular features of the disease and with blood parameters. For the patients with IPF, expression of miR-153 showed no significant association with family history of the disease ($p = 0.841$) and with categorical disease severity ($p = 0.104$) either. However, correlation matrices showed significant inverse correlation of miR-153 levels with lymphocyte counts ($r = –0.493$, $p = 0.001$) and also with FVC %, confirming the hypothesis that physiologic decline is reflected in the molecular downregulation of this miRNA.

There were also positive associations seen between miR-153 and neutrophils which suggest that active inflammatory cells may alter the profile of circulating miRNAs. These results suggest that in IPF, repressed miR-153 may be both a byproduct of and a contributor to the perpetuation of pro-fibrotic inflammatory mechanisms. Collectively, these observations indicate that miR-153 may be a candidate molecular link between inflammation and fibrosis, which provides a mechanistic basis for its proposed role as a biomarker (Table 4).

Table 4: Correlation between miR-153 gene expression and selected clinical and hematological parameters in patients with idiopathic pulmonary fibrosis.

Parameter	r	p-value	Interpretation
White blood cell (WBC)	0.080	0.543	NS – no significant correlation
Neutrophils (count)	0.232	0.075	Weak positive, NS
Lymphocytes (count)	-0.493	0.001 *	Moderate negative correlation
Neutrophils (%)	0.341	0.008 *	Significant positive correlation
FVC (%)	-0.613	0.001 *	Strong negative correlation
FEV_1/FVC (%)	0.176	0.178	NS – no significant correlation

Discussion

This study marks the first reported case of the significant down-regulation of circulating miR-153 in IPF patients, compared to the healthy population, which may serve to strengthen the case for this molecule being a non-invasive biomarker for the activity of the disease. This finding supports previous observations of dysregulated miRNAs facilitating fibrotic remodeling by influencing epithelial-mesenchymal transition (EMT), fibroblast activation, and extracellular-matrix (ECM) (11, 12). Some miRNAs, such as miR-29 and the miR-200 family, and the TGF-β pathway interact to regulate fibroblast collagen expression (11). In the same manner, Kaur et al. (2021) demonstrated that exosomal miRNA profiles derived from bronchoalveolar lavage fluid can differentiate IPF from other forms of lung disease, indicating systemic miRNA changes as in the present study (12). These studies and the present observations suggest that miR-153, like other members of this fibrotic regulatory network, is underexpressed, which enhances fibroblast activity in IPF.With regard to the current study's findings, the substantial drop in miR-153 expression down-regulation exhibited strong negative correlation with lung-function indices, particularly FVC %, demonstrating that the absence of this regulatory miRNA comes with physiological declines. Kadota et al. (2020) demonstrated that fibroblast-derived extracellular vesicles can transmit pro-fibrotic signals leading to epithelial senescence (13). This supports the interpretation that diminished circulating miR-153 reflects lowered antifibrotic control over fibroblast-epithelium vesicle crosstalk. Zhou et al. (2021) also demonstrated that exosomal miRNA therapy targeting Wnt5a alleviated experimental fibrosis in mice (14). Zhou et al. (2021) observed that the re-introduction of antifibrotic specific miRNAs capable of restoring epithelial homeostasis, extended therapeutic potential. This is in line with the current findings that suggest substantial antifibrotic therapeutic control may be readily accessible, reinforcing the diagnostic potential of this work (14).Insights revealed from this study that is heightened neutrophils and decreased lymphocytes helps

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evidence new connections inflammation and miRNA modulation. Wu et al. (2020) demonstrate that miR-30a diminishes TGF-β1-induced fibroblast activation and, in turn, inflammatory mediators and ECM (Wu et al. 15). Likewise, Li et al. (2021) detailed the mechanisms through which miR-184 diminishes proliferation and EMT in airway epithelial cells via the TP63 gene and its application to IPF (Li et al. 16). My results are consistent with those Li et al. (2021) in that loss of certain protective miRNAs such as miR-153 might promote unregulated EMT and fibrotic signaling in the more advanced stages of the disease in the lungs (Li et al. 16). Pivotal to all studies is the idea that the absence of repression of certain miRNAs in IPF is a multifaceted, integrative change in the gene expression constellation that favors pro-fibrotic mechanisms.Moreover, microarray-based analysis by Li et al. (2020) showed unique miRNA signatures that classify IPF samples with a high degree of accuracy, which corroborates their application as potential diagnostic biomarkers (17). The present data showing 100% sensitivity and specificity for miR-153 expression strengthens those results even further. Similarly, Negrete-García et al. (2022) noted that exosomal miRNAs function as intercellular signals in IPF, facilitating fibroblast–epithelial signaling loops that enhance fibrogenesis (18). The current finding aligns with those by Negrete-García et al. (2022) as decreased circulating miR-153 may be due to sequestration of this miRNA within profibrotic vesicles, thereby reducing its regulatory availability in circulation (18). These consistent findings underscore miR-153 as a mechanistic and a diagnostic component in the pathobiology of IPF.The emergent literature linking dysregulated miRNA in IPF to other fibrotic or infectious lung conditions is quite startling. As mentioned in the work of Guiot et al. (2023), there are overlapping miRNA signatures in COVID-19 and IPF and these include inflammation and fibrosis pathways (19). Soccio et al. (2023) also emphasized the importance of determining airway biomarkers that predict progression of disease, which is consistent with our emphasis on miR-153 as a progression marker (20). This finding is in agreement with those of Soccio et al. (2023) in that miRNA biomarkers can aid in the early detection of progressive pulmonary fibrosis which is not captured by classical physiologic metrics (20). The pervasive downregulation of miR-153 in the entire cohort and the association of clinical severity suggest that miR-153 should be a candidate biomarker in the diagnosis of IPF as well as monitoring of therapy.

Conclusion

The study at hand establishes for the first time the clinically relevant relationship between the marked lower level of circulating miR-153 and the functional and inflammatory derangements in patients suffering from idiopathic pulmonary fibrosis (IPF). Its pronounced association with disease severity, pulmonary fibrosis, and decreased lung capacity indicates that it could serve as a valuable non-invasive prognostic and progress assessment biomarker in IPF. Moreover, its prominent association with the disease severity and pulmonary restriction indicates that it might have a regulating fibrosis-promoting inflammatory and epithelial–mesenchymal pathway. Its potential diagnostic value as a prognostic and progress marker should be analyzed in combination with other prognostic tools on IPF for better stratification and individualized care of patients. Its diagnostic value as a progress marker would be clinically relevant. Importantly, it could also be a novel target for antifibrotic therapy restoration.

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Conflict of interest

The authors declare no conflict of interest related to this study.

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