

Study of Electrocardiographic Abnormalities in Patients with Chronic Obstructive Pulmonary Disease and its Correlation With Severity

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Abstract

Introduction: The current study aimed to identify the electrocardiographic (ECG) changes in chronic obstructive pulmonary disease (COPD) patients and their correlation to severity.

Methods: A prospective observational study was conducted on 100 patients with COPD with expiratory volume in the first second (FEV1) < 0.7. Patients were categorized according to GOLD criteria and underwent Spirometry and ECG.

Results: Among the COPD cases, 40% belonged to severe airflow limitation followed by very severe (32%), moderate (22%), and mild (6%) categories. The mean forced FEV1% in mild, moderate, severe, and very severe COPD was 81.3±0.57, 63.9±6.28, 41.15±4.59, and 25.625±2.41, respectively ($P < 0.05$). The higher risk factor for COPD is smoking in 80% with a mean pack of 20.67±6.5 years. The most ECG abnormality in COPD patients was right ventricular hypertrophy (RVH) (52%), followed by right bundle branch block (RBBB) in 40% of patients, right axis deviation (34%), P pulmonale (32%), and atrial fibrillation in 22% of COPD cases, respectively. Furthermore, there was a strong association between ECG abnormalities and COPD severity. COPD patients with a poor FEV1/FVC% ratio had more ECG abnormalities, and the ECG abnormalities were shown to be highly correlated with the severity, duration, and length of symptoms.

Conclusion: COPD is a common condition in patients with a smoking history of >20 pack-years, being more common in males in their 5th decade of life. Moreover, low FEV1 values were associated with ECG abnormalities. As the duration/severity of the disease rises, ECG abnormalities become more common. To avoid cardiac mortality and morbidity, all COPD patients should undergo cardiac examination through ECG for the management of co-morbidities.

Keywords: Chronic obstructive pulmonary disease, Electrocardiography, Right ventricular hypertrophy

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined as “a stage of disease with irreversible airflow limitation”,¹ which includes emphysema, chronic bronchitis, and small airway disease.^{2,3}

The World Health Organization (WHO)/National Heart, Lung, and Blood Institute defined airflow blockage as a forced expiratory volume in the first second (FEV1) and FVC ratio of <0.70, also projected by GOLD. When FEV1 is >80% of prediction, 50%-80% of prediction, 30%-50% of prediction, and lower than 30% of prediction, the severity of airflow restriction was rated as stage 1 or mild,

stage 2a or moderate, stage 2b or severe, and stage 3 or very severe, respectively.^{4,5} COPD is currently the sixth-leading cause of mortality, but it is expected to be the third prevalent cause of death. After pulmonary tuberculosis, COPD is the second most common disease affecting the respiratory system in India.⁵ More than 3 million people died due to COPD in 2012, accounting for 6% of all deaths worldwide.⁶

Many investigations have shown that COPD has considerable systemic consequences, the most prevalent of which is cardiac.^{7,8} Cardiovascular illness accounts for 20%-25% of all deaths in COPD patients with advanced



disease. COPD develops pulmonary hypertension, Cor pulmonale, right ventricular dysfunction, and left ventricular dysfunction by affecting the pulmonary blood vessels, right ventricle, and left ventricle.⁹

Various processes contribute to the electrocardiographic (ECG) changes that accompany increasing airway obstruction in COPD, including (a) lung hyperinflation, which may alter transmission or cardiac action in the current circumstances, (b) diaphragm depression, which may change the heart anatomic connection to the electrode placements, and (c) pulmonary hypertension, which is caused by vasoconstriction.

Airflow obstruction is characterized as mild (Gold 1), moderate (Gold 2), severe (Gold 3), or very severe (Gold 4) based on FEV1.¹⁰ Comorbidities can also arise in patients with mild, moderately severe, or very severe airflow limitation and demand specific therapy.^{11,12} By taking all these considerations into account, the current study was designed to assess the ECG changes in COPD patients and their association with severity at our tertiary care center. In other words, this study aimed to analyze the ECG changes in COPD patients and their association with COPD severity.

Patients and Methods

Subjects

A cross-sectional study was carried out on 100 patients who attended the Department of General Medicine and Pulmonology.

Sample Size Calculation

A nonprobability consecutive sampling technique was used. The sample size is calculated by assessing a 95% confidence interval (1-a). The expected population proportion (P) was 8%, and the absolute precision (d) was 4.5%.

The following formula was used:

$$N = Z^2 \times (P) \times (1-P) / d^2,$$

where Z indicates the Z value (e.g., a 95% confidence interval of 1.96), d is the absolute precision of 5% (0.05), and P is the prevalence of the disease (N = 100).

Inclusion Criteria

Diagnosed cases of COPD based on symptoms and confirmed by radiographic and pulmonary function tests were included.

Exclusion Criteria

Cases with bronchial asthma, bronchiectasis, pulmonary tuberculosis, known congenital or acquired heart diseases, diabetes mellitus, and hypertension were excluded.

Data Collection

After getting approval from the Institutional Ethical

Committee, prior consent of all patients who were diagnosed to have COPD fulfilling inclusion criteria during the study period who attended OPD and were admitted to wards in the Department of General Medicine and Department of Pulmonary Medicine was taken. Data related to History, ECG, pulmonary function tests, chest x-ray, and routine blood laboratory investigation were collected.

Procedure

First, 12-lead ECG was recorded for 10 minutes after the rest of the supine, 10 mm/mV of gain, and default filter settings. Then, all findings were interpreted by the researcher under the supervision of a cardiologist.

General Electrocardiographic Abnormalities

- Right atrial enlargement (P wave amplitude in leads II, II, and augmented vector foot [aVF] of >2.5 mm or V1 of 21.5 mm),
- Right ventricular hypertrophy (RVH) (R in V1: >7 mm, R/S in V1: >1, or ventricular activation time in V1: >35 ms),
- Clockwise rotation (R/S ratio in V5: <1),
- Low voltage limb leads (QRS [R+S]: <5 mm in I, II, aVF, and III),
- QS complex (if present in lead III),
- Right axis deviation (RAD) (>90°),
- Left axis deviation (<-30° to -90°),
- Prolonged QT interval (>0.44 s),
- Atrial fibrillation (>3 saw-shaped waves between 2 QRS complexes in lead II),
- T-wave changes (>1 mm depression below or >5 mm elevation above baseline),
- ST depression (>1.5 mm below baseline).

Electrocardiographic Criteria

All values were recorded in the pre-approved proforma. ECG criteria for RVH:

1. RAD of QRS
2. P pulmonale
3. R < S in V6.
4. A + R- PL > 0.7; A = maximal R/Amplitude in V1 or V2, R = maximal S in lead I or V6, PL = minimum S in V1 or minimal R in lead I or V6.

Electrocardiographic Criteria for Cor Pulmonale

1. RAD of QRS complex,
2. P pulmonale,
3. With rSR in right precordial leads and QRS duration >0.12 seconds, the right bundle branch block (RBBB) (incomplete) occurs.
4. R/S-ratio in V1 >1. Dominant 'R' wave in right precordial leads,
5. R/S ratio in V6 <1.

RV dilatation and strain have been used to explain the

inversion of the 'T' wave in the right leads. Hypoxia-related generalized T wave inversion could be a nonspecific condition. Then, spirometry was performed on patients who meet the inclusion and exclusion criteria.

Statistical Analysis

The collected data were entered into the Excel spreadsheet and transferred to the SPSS version 23 for statistical analyses (IBM, Chicago, Illinois). Quantitative variables were presented as mean and standard deviation, and averages of quantitative variables were compared using one-way analysis of variances and categorical variables by either the chi-square test or Fisher's exact test. The *P* value for all tests was considered significant at <0.05 .

Results

Baseline Characteristics

This study reported mild (6%), moderate (22%), severe (40%), and very severe (32%) disease of COPD cases, respectively. Variables such as age, gender, and body mass index of COPD cases did not show significant differences between the COPD groups.

The mean age was 65.26 ± 9.5 years with an age range of 41-84 years. All males were smokers, with 60% of them smoking Beedi and Chutta, 16% smoking cigarettes, and 6% smoking both. Females were exposed to biocombustibles. The most common X-ray abnormality was an emphysematous chest in 68% of patients, followed by tubular heart in 60%, flattened diaphragm in 58%, and

prominent pulmonary conus in 42%.

Pulmonary Function Test Variables

The mean FEV1% in mild was 81.3 ± 0.57 , in moderate was 63.9 ± 6.28 , in severe was 41.15 ± 4.59 , and in very severe was 25.625 ± 2.41 . The overall mean FEV1% was $43.6 \pm 17.48\%$ which was statistically significant ($P=0.001$), as depicted in Table 1. As Tables 2 and 3 illustrate, 34% of COPD patients exhibited FEV1/FVC ratio of 21%-40%, 42% of patients indicated FEV1/FVC ratio of 41%-60%, and 24% of patients showed FEV1/FVC ratio of 61%-70%.

Electrocardiographic Changes

ECG changes were observed to be 33.33%, 45.45%, 55%, and 93.75% in mild, moderate, severe, and very severe COPD cases. The most common ECG abnormality was RVH (52%), followed by RBBB (40%), RAD (34%), P pulmonale (32%), and atrial fibrillation (22%). Furthermore, there was a strong association between ECG readings and the severity.

The incidence of P pulmonale, RAD, RVH, and RBBB showed significant association with % FEV1 severity. Most of the patients (40%) were in GOLD stage III, and RVH (52%) was the most prevalent ECG abnormality. Furthermore, the most common ECG changes were P wave axis $> +90^\circ$ in 54% of patients, followed by QRS axis $> +90^\circ$ in 40%, P wave height > 2.5 mm in lead II in 38% of cases, and RV1 height > 7 mm in 28% of cases,

Table 1. Demographics, Duration of Illness of COPD, Radiological Findings, and ECG Changes in Study Population

Characteristics	Gold 1 Mild (n=6)	Gold 2 Moderate (n=22)	Gold 3 Severe (n=40)	Gold 4 Very Severe (n=32)	P Value
Mean age	53.3 \pm 7.09	60.45 \pm 8.3	66.9 \pm 9.09	68.75 \pm 8.73	0.12
Males/female	4/2	16/6	32/8	28/4	0.47
BMI	22.3 \pm 4.04	21.9 \pm 5.37	22.1 \pm 2.53	20.3 \pm 1.36	0.75
Smoking (pack/years)	20.67 (5.86)	19.72 (17.54)	22.88 (6.17)	21.47 (8.63)	0.419
FEV1 (%pred, SD)	81.3 (0.57)	63.9 (6.28)	41.15 (4.59)	25.62(2.41)	<0.001
Duration of illness of COPD					
Years, No. (%)					
<5	6 (100)	6 (27.27)	6 (15)	12 (37.5)	30 (30)
5-10	0 (0)	8 (36.36)	16 (40)	12 (37.5)	36 (36)
10-15	0 (0)	4 (18.18)	12 (30)	4 (12.5)	20 (20)
>15	0 (0)	4 (18.18)	6 (15)	4 (12.5)	14 (14)
Radiological Findings in COPD Cases					
Chest x-ray, No. (%)					
Emphysema	4 (66.67)	18 (81.82)	28 (23.3)	18 (56.25)	68 (68)
Cardiomegaly	0 (0)	2 (9.09)	12 (30)	12 (37.5)	26 (26)
Prominent pulmonary conus	0 (0)	2 (9.09)	16 (40)	24 (75)	42 (42)
Tubular heart	2 (33.33)	16 (72.72)	24 (60)	18 (56.52)	60 (60)
Flattened diaphragm	2 (33.33)	16 (72.72)	22 (55)	18 (56.25)	58 (58)
ECG changes with disease severity of COPD	2 (33.33)	10 (45.45)	22 (55)	30 (93.75)	64 (64)

Note. ECG: Electrocardiographic; COPD: Chronic obstructive pulmonary disease; BMI: Body mass index; Pred: Prediction; FEV1: Forced expiratory volume in the first second; SD: Standard deviation.

respectively (Table 4).

Regarding ECG change, RVH was detected in 33%, 36%, 50%, and 68% of mild, moderate, severe, and very severe category patients. In addition, P. pulmonale was found in 9.09%, 20%, and 68% of moderate, severe, and very severe patients, and RAD was found in 9%, 50%, and 37% of moderate, severe, and very serious patients. Furthermore, poor R wave progression was noted in 9%, 20%, and 56% of

moderate, severe, and very severe category patients. RBBB was observed in 18.18%, 35%, and 68% of moderate, severe, and very severe patients, respectively.

Patients with moderate, severe, and very severe categories had atrial fibrillation of 18%, 15%, and 37%, respectively. Further, there was a strong association between these ECG anomalies and the various COPD illness groups (Table 5).

Table 2. The Distribution of FEV1% among COPD Patients

COPD	FEV1%		P value
	Range	Mean ± SD	
Mild (n=6)	81-82	81.3 ± 0.57	< 0.001*
Moderate (n=22)	51-74	63.9 ± 6.28	
Severe (n=40)	32-49	41.15 ± 4.59	
Very severe (n=32)	21-29	25.625 ± 2.41	
Total	21-82	43.6 ± 17.48	

Note. COPD: Chronic obstructive pulmonary disease; SD: Standard deviation.

* Significant.

Discussion

The age of the patients in this study ranged between 41 to 84 years, with the mean age of 65.26 + 9.525 years. COPD is mostly a disease associated with the elderly, and age is a risk factor for the onset of the disease. In this study, the male to female ratio was 4:1. Due to higher exposure to smoking, there was a male preponderance in every research. This corroborates the studies by Verma et al,¹³ Katiyar and Khare,¹⁴ and Mitsiki et al.¹⁵

Twenty female patients with COPD symptoms and signs were included in the current study. Even though

Table 3. The Distribution of ECG Findings

ECG Changes	GOLD 1 Mild (n=6)		GOLD 2 Moderate (n=22)		GOLD 3 Severe (n=40)		GOLD 4 Very Severe (n=32)		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
P Pulmonale	0	0	2	9.09	8	20	22	68.7	32	32
Poor R wave progression	0	0	2	9.09	8	20	18	56.25	28	28
RAD	0	0	2	9.09	20	50	12	37.5	34	34
Low voltage ECG (%)	0	0	4	18.18	14	35	22	68.75	40	40
RVH	2	33.33	8	36.36	20	50	22	68.75	52	52
RBBB	0	0	4	18.18	14	35	22	68.75	40	40
S1Q3	0	0	2	9.09	4	10	6	18.75	12	12
S1S2S3	0	0	4	18.18	14	35	22	68.75	40	40
Atrial fibrillation	0	0	4	18.18	6	15	12	37.5	22	22

Note. ECG: Electrocardiographic; RAD: Right axis deviation; RVH: Right ventricular hypertrophy; RBBB: Right bundle branch block.

Table 4. The Association between ECG Changes and Spirometry Severity Grades

ECG Changes	Criteria	Number (N=100)	Spirometry-severity Grad				P Value
			GOLD 1 Mild (n=6)	GOLD 2 Moderate (n=22)	GOLD 3 Severe (n=40)	GOLD4 Very Severe (n=32)	
P wave axis	< 90°	46	4 (66.66)	14 (63.63)	20 (50)	8 (25)	0.182
	≥ + 90°	54	2 (33.34)	8 (36.63)	20 (50)	24 (75)	
QRS axis	< 90°	60	2 (33.34)	14 (63.63)	24 (60)	20 (62.5)	0.80
	≥ + 90°	40	4 (66.66)	8 (36.63)	16 (40)	12 (37.5)	
P wave height in lead II	< 2.5	68	2 (33.34)	15 (68.18)	27 (67.5)	24 (75)	0.485
	≥ 2.5	32	4 (66.66)	7 (31.81)	13 (32.5)	8 (25)	
RV6 height in mm	> 5.0	60	2 (33.34)	14 (63.63)	24 (60)	20 (62.5)	0.803
	< 5.0	40	4 (66.66)	8 (36.63)	16 (40)	12 (37.5)	
RBBB	Absent	60	6 (100)	18 (81.81)	26 (65)	10 (31.25)	0.043*
	Present	40	0	4 (18.18)	14 (35)	22 (68.75)	
RV1 height in mm	< 7	72	2 (33.34)	14 (63.63)	28 (70)	28 (87.5)	0.207
	> 7	28	4 (66.66)	8 (36.63)	12 (30)	4 (12.5)	

Note. ECG: Electrocardiographic; RBBB: Right bundle branch block.

* Significant.

Table 5. The Comparison of ECG Change Mean Values of FEV₁

ECG Changes	Criteria	Number (N=100)	FEV1 (Mean±SD)	P value
P wave axis	<90°	46	1.32±0.35	0.002*
	≥+90°	54	1.04±0.28	
QRS axis	<90°	60	1.28±0.33	0.002*
	≥+90°	40	0.94±0.24	
P wave height in mm	<2.5	68	1.32±0.30	<0.001*
	≥2.5	32	0.87±0.17	
RV6 height in mm	>5.0	60	1.25±0.34	<0.001*
	<5.0	40	0.89±0.17	
RBBB	Absent	60	1.16±0.34	<0.001*
	Present	40	0.86±0.00	
RV1 height in mm	<7	72	1.15±0.34	0.279
	>7	28	1.05	

Note. ECG: Electrocardiographic; RAD: Right axis deviation; SD: Standard deviation; RBBB: Right bundle branch block.

* Significant.

none of the women were smokers, they were all exposed to fuel smoke. Cooking is typically done using wood and cow dung in this part of the population. This could be a significant risk factor for the development of COPD in female patients.

The most major risk factor for COPD was smoking in 80% of COPD patients. Smoking is the most frequent etiological risk factor for COPD, according to Jindal et al.¹⁶ The average pack years of smoking in this study was 20.67 ± 6.5 years. Most of the patients had a 20 pack-year smoking history, and 60% of patients have smoked for 20 to 25 years. In the study by Jatav et al,¹⁷ the largest number of pack years was between 20 and 29 years. In the study by Chaudhari and Shrimali,¹⁸ 41.4% of the patients were between the ages of 11 and 20 pack years. In the current study, 12% of the patients were non-smokers, that is, exposure to bio combustibles and occupational exposure was present, and all of them were females.

In the current study, all patients had shortness of breath which was graded according to the modified medical research council. Further, 80% of the patients had a cough and sputum which is in line with Kutum et al.¹⁹ Other commonly associated symptoms were wheezed in 52% of patients and chest discomfort in 44% of patients.

The mean value for FVC, FEV₁, FEV₁/FVC% in the present study was 2.27 ± 0.52, 1.15 ± 0.34, and 48.1 ± 12.92, respectively, which is comparable to the study group in Kamat et al.²⁰ Most of the patients belonged to moderate airflow limitation. In the current study, the predicted mean ± standard deviation FEV₁% was 43.6 ± 17.48% which corresponded to the mean FEV₁% of 45 ± 15.8% noted in Huang et al.²¹

In our study, the majority of patients belonged to severe airflow limitation, and the mean ± standard deviation of FEV₁% was 81.3 ± 0.57, 63.9 ± 6.28, 41.15 ± 4.59, and

25.625 ± 2.41% in Gold 1, Gold 2, Gold 3, and Gold 4, respectively. This corroborated Venkateswara Rao and Eswaramma,²² where the mean FEV₁ was 71.1 ± 9.3%, 43.2 ± 6%, and 24.4 ± 6.3%, in the moderate, severe, and very severe groups, respectively.

Based on GOLD guidelines, the number of mild, moderate, severe, and very severe COPD patients in the present study was 6%, 22%, 40%, and 32%, respectively, whereas a study by Verma et al¹³ reported that the frequency of mild, moderate, severe, and very severe disease was 5.8%, 34.78%, 36.23%, and 23.18%, respectively.

The profile signs of cor pulmonale were consistent with the study conducted by Venkateswara Rao and Eswaramma.²² In our study, 68% of the total patients had hyperinflation of lung fields, 26% of the patients had cardiomegaly, 58% had flattened domes of a diaphragm, and 60% had a tubular heart. Moreover, prominent pulmonary conus was present in 42% of the patients, and the radiological findings were significantly associated with the severity of COPD. Additionally, all patients of GOLD 4 had prominent pulmonary conus which is evident in the chest x-ray.

When the ECG abnormalities were associated with the length of symptoms, P pulmonale, RAD, RVH, and partial RBBB all increased with the duration of the condition, however, only RAD had statistical significance. The most frequent observed ECG changes were P wave axis ≥ +90° (54%), QRS axis > +90° (40%), P wave height ≥ 2.5 mm in lead II (38%), R wave in V₆ ≤ 5 mm (28%), and R/S ratio in V₅V₆ ≤ 1 (28%). Moreover, 14 patients had R wave in V₁ > 7 mm (28%) and RBBB (40%), whereas the study by Gupta et al²³ showed that the most frequent observed ECG changes were P wave axis ≥ +90° (64%), QRS axis > +90° (40%), P wave height ≥ 2.5 mm in lead II (38%), R wave in V₆ ≤ 5 mm (28%), and R/S ratio in V₅V₆ ≤ 1 (26%), R wave in V₁ > 7 mm (2%), and RBBB (4%).

ECG alterations were found in most individuals as the severity of airflow obstruction increased. As previously stated, hypoxia is a primary contributor to ECG abnormalities in COPD patients. In COPD, hypoxia is related to the severity of airway obstruction. In this study, there was a strong negative connection between ECG alterations and an FEV₁/FVC ratio spirometry assay.

In this study containing 100 patients, RAD was observed in 34% cases. Furthermore, 52% of patients had RVH (R/S in v₁ > 1 mm and R/S in V₆ 1 mm), 32% patients had P pulmonale, and only a handful of them exhibited atrial fibrillation (22%) and low voltage complexes (40%).

The P pulmonale configuration was higher in the severe group (75%). This corroborated Chaudhari and Shrimali's study¹⁸ where it was 54.8% in the severe group. In the study conducted by Sruthi et al on 120 patients,²⁴ the most common abnormality was P pulmonale (37.7%) which was similar that in the current study. RVH was found in 24.59% of the patients which was less than that in the

present study. Additionally, RBBB was found in 9.84% of patients, which is almost similar to the present study. RAD was also present in 19.67% and was around 45% in the severe group. Although the overall percentage of RAD was 41.6 % in the current study, it was 66.6 % in the very severe group. Hence, all studies have demonstrated that the right ventricular and right atrial changes were quite high in severe and very severe groups ($P=0.001$).

The presence of P pulmonale in the ECG was associated with the severity of COPD. It showed variable percentages in various other studies such as those conducted by Banker and Verma (35%)²⁵ and Alexander et al (52.5%).²⁶ This variation is due to the variation in the severity of COPD in different studies.

In the study by Sarath Kumar Reddy et al,²⁷ atrial ectopics, ventricular ectopics, and atrial fibrillation were seen in more than 50% of the patients, while atrial fibrillation in our study was 13.3%, where 22.2% of the patients were in the very severe group.

In the study by Gupta et al,²³ P pulmonale and right ventricular changes were more common. Furthermore, there was a significant negative association between FEV1 and ECG changes. According to Mod et al,²⁸ the cor pulmonale alterations were caused by the vertical alignment of the heart caused by RVH, and ECG can be also used as a screening tool to detect the changes in the P pulmonale in COPD patients.

In the current study, 32% of the study population had P pulmonale. Gupta et al²⁹ identified P pulmonale in 17.5% of cases, and 20% of the study population in Yaksic et al³⁰ had P pulmonale. Kaushal et al³¹ reported that 32% of the study sample have P pulmonale, but this figure was much higher (62%) in the study by Jatav et al.¹⁷ As a result, ECG and ECHO examinations are required for all COPD patients as they contribute to the early detection and management of heart problems.

In our study, 33% of patients in the mild category exhibited ECG alterations, 45% in the moderate category, 55% in the severe category, and 93% in the very severe category. Padmavati and Raizada³² and Jatav et al¹⁷ found comparable results. Patients in the mild category had no atrial fibrillation, but those in the moderate, severe, and very severe categories had 18%, 15%, and 38% atrial fibrillation, respectively. These ECG anomalies were found to have a substantial association with COPD disease categories. Padmavati and Raizada³² as well as Jatav et al¹⁷ found comparable results. According to the findings, the severity of problems increases in lockstep with the severity of COPD, forming a linear relationship, and changes in the ECG were shown to be highly associated with the severity of the condition.

Conclusion

COPD individuals with a higher degree of severity had more ECG alterations. There was a strong association

between ECG findings and the severity. Hence, the early detection and treatment of cardiac co-morbidities would lower COPD patient mortality. Accordingly, preliminary spirometry screening of COPD patients and documentation of their FEV1% with frequent and regular monitoring could be an essential technique in preventing or delaying heart disease events and thereby lowering patient mortality.

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Authors' Contribution

Conceptualization: Swarna Deepak Kuragayala.

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Competing Interests

All authors declared no potential conflict of interests regarding the research, its authorship, and its publication.

Ethical Approval

This protocol was approved by the institutional Ethics Committee (No. IEC/2018/01).

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