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Original Article

Comparative analysis of pulmonary functions, inflammatory and oxidative stress biomarkers in smoker and biomass smoke exposed COPD

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Abstract

Introduction: Non-smoking chronic obstructive pulmonary disease (COPD) is one of the major contributors among total COPD cases in low- and middle-income countries. This study aimed to investigate pulmonary functions and estimate systemic and airway inflammatory and oxidative stress markers in serum and exhaled breath condensate (EBC) of cigarette-smoking COPD and biomass smoke-exposed COPD patients, comparing them with healthy smokers and healthy non-smokers.

Methods: A total of 45 participants were enrolled: smoker COPD (n = 10), biomass smoke-exposed COPD (n = 10), smoker control (n = 10), and non-smoker control (n = 15). Pulmonary function tests, including spirometry and impulse oscillometry, were performed. Inflammatory and oxidative stress marker levels in both serum and EBC were estimated.

Results: Spirometric parameters, including slow vital capacity (SVC), forced expiratory volume at 1st second (FEV1), forced vital capacity (FVC), and FEV1/FVC were significantly less in COPD groups (smoker/biomass) as compared to controls. Smoker COPD had less FEV1/FVC than biomass-exposed COPD. COPD groups (smoker/biomass) exhibited significant impairment in lung mechanics, characterized by increased peripheral airway resistance (R5-R20), reactance at 5 Hz (X5), and resonant frequency (Fres), indicating involvement of peripheral airways. However, no significant change in lung mechanics exists between smokers' COPD and biomass-exposed COPD. Among the oxidative stress markers, 8-isoprostane and nitrotyrosine-3 (NT3) levels in EBC were significantly higher in smoker-COPD compared to biomass-exposed COPD and non-smoker controls, respectively.

Conclusion: Significant pulmonary function impairment was observed in both smoker COPD and biomass smoke-exposed COPD. Inflammatory and oxidative stress markers are more deranged in smoker COPD than in biomass smoke-exposed COPD and healthy controls.

Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, expectoration) due to persistent abnormalities of the airways (bronchitis, bronchiolitis) and alveoli (emphysema) that often result in progressive airflow limitation.[1] It has multiple etiological factors, clinical phenotypes, and co-morbidities. COPD is the third leading cause of death worldwide, causing 3.23 million deaths in 2019.[2] According to the World Health Organization Global Status Report, an estimated smoking prevalence is expected to reach 1.6 billion by 2025.[3] Although tobacco smoking is the major environmental risk factor for COPD, only a subset of smokers develop COPD.[4] So, other factors like exposure to biomass fuel,[5] environmental pollution,[6] passive smoking, and genetic elements may also contribute to the development of the disease. About 3 billion people are exposed to smoke from biomass fuel compared with 1.01 billion people who smoke tobacco. The burden of non-smoking COPD is much higher than previously believed, as an estimated 25%-45% of patients with COPD have never smoked.[7] Non-smoking-mediated COPD now contributes to over 50%of the global burden of COPD.[8]

Biomass comprises a group of biological materials, such as living organisms, animals, and vegetables, which produce large amounts of smoke/particulate matter and NO2 in closed or poorly ventilated areas after combustion. Some of these materials, such as wood, cow dung, shell, coir of coconut, and crop residues from agriculture, are used as

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fuel for cooking in developing countries.[7,9] Approximately 50% of the world's population in India, China, Bangladesh, and sub-Saharan Africa use biomass fuels. Exposure to biomass fuel smoke is responsible for diseases like COPD and bronchial carcinoma.[10,11] Biomass smoke induces an inflammatory response and tissue damage in the airways and also causes irreversible obstructive airway disease in genetically primed individuals.[12,13]

Increased inflammatory markers and abnormally low pulmonary function test findings were reported in both biomass smoke exposure and cigarette smoke exposure groups. At least 18 years of biomass smoke exposure was reported to be sufficiently high to be responsible for obstructive and restrictive pulmonary diseases.[14] Several studies have reported that women using solid biomass fuel (SBF) and mixed fuel had a relatively high prevalence of phlegm, cough, and eye irritation as compared to liquefied petroleum gas (LPG) users, and it was associated with significantly lower FEV1values in SBF users.[15-17]

Inflammation and oxidative stress are two critical factors associated with the pathophysiology of COPD. An increased number of alveolar macrophages, neutrophils, T lymphocytes, and, in some patients, eosinophils characterize the inflammation observed in COPD. All these cells, along with structural cells like epithelial cells, endothelial cells, and fibroblasts, secrete various pro-inflammatory mediators, including cytokines, chemokines, growth factors, and lipid mediators. Systemic inflammation is also observed in patients with COPD and can worsen co-morbidities such as cardiovascular diseases, diabetes, and osteoporosis.[18]

Oxidative stress is key in driving COPD-related inflammation, even in ex-smokers. Lungs are exposed to pollution and cigarette smoke, which generate oxidants. To oppose their potential deleterious effects, living organisms, particularly the lungs, have developed a sophisticated antioxidant system comprising several molecules, especially glutathione (GSH) and protein-cys-SH (PSH). GSH is one of the most effective enzymatic antioxidants, and its activity is an essential feature in determining oxidative damage. PSH's antioxidant properties protect biological systems against oxidative stress.[19,20] Increased oxidative stress and protease to anti-protease imbalance occur in COPD and are involved in disease initiation and progression. Oxidative stress occurs when ROS are produced in excess of the antioxidant defence mechanisms, resulting in harmful effects, including damage to lipids, proteins, and DNA transcription factors and enzymes, promoting fibrosis and potentiating the effects of cigarette smoke and biomass smoke on lungs.[21,22] Oxidative stress also causes corticosteroid resistance through reduced expression of histone-deacetylase-2.[23]

Even though spirometry is used for diagnosing and prognosing COPD, its ability to diagnose small airway disease is still debatable.[24] On the other hand, the impulse oscillometry system has been used successfully to measure both central and peripheral airway resistance and its relationship with health status and dyspnea in patients with COPD.[25,26] Various biomarkers associated with the disease provide information about the severity of the disease and acute exacerbations. They also act as a valuable tool to assess the treatment's effectiveness and help personalize it. Bronchoscopy and bronchoalveolar lavage are the most reliable techniques for estimating airway inflammation. However, they are invasive and cannot be repeated multiple times to monitor the disease course. During recent years, there has been a growing interest in using non-invasive techniques like the collection of exhaled breath condensate (EBC) to study and monitor airway inflammation, remodelling, and oxidative stress in patients having asthma and COPD.

Different biomarkers secreted by airway epithelial cells and inflammatory cells are present in airway lining fluid (ALF). EBC consists of water vapour and aerosolized particles generated from ALF, but its composition is similar to that of ALF. Also, the sensitivity of detecting various biomarkers makes EBC a novel and potentially important diagnostic tool.[27] Various biomarkers can be studied in EBC, like small inorganic compounds (H2O2, pH, and nitric oxide-related biomarkers), lipid mediators (8-isoprostane, leukotrienes, and prostaglandins), and small proteins (cytokines, chemokines, and nucleic acid derivatives).[28]

Studies investigating the biomarkers of inflammation and oxidative stress in EBC and their correlation with airway obstruction and resistance are still lacking for smokers with COPD and biomass smoke-exposed COPD patients. In this study, we measured lung volumes, capacities, respiratory impedance, and estimated airway inflammation and oxidative stress in smoker-COPD and biomass-smoke-exposed COPD patients by collecting and analysing EBC.

Methods

Selection of Subjects

It is a cross-sectional observational study, and the protocol was reviewed and approved by the Institute Ethics Committee, AIIMS, New Delhi. Written informed consent was obtained from all the participants before the study. Smoker COPD (n = 10) and biomass smoke-exposed COPD (n = 10) patients were recruited from the Pulmonary, Critical Care, and Sleep Medicine outpatient clinic. Age-matched healthy controls (smoker controls (n = 10) and non-smoker controls (n = 15)) were also recruited. The COPD patients were grouped by history taking; patients with a history of smoking for more than ten packs/year were recruited as smoker COPD. Patients with a history of daily wood smoke exposure for at least 200 hours/year were recruited as biomass smoke-exposed COPD. Healthy smokers with no history of biomass exposure were recruited as smoker controls. Subjects

with a history of fever or respiratory tract infection in the past month, evidence of cardiovascular, musculoskeletal, chronic immunological diseases and inflammatory disorders, or taking oral steroids and antibiotics over the past four weeks were excluded from the study. All subjects' weight (kg) and height (cm) were recorded. After recruitment, subjects underwent spirometry and impulse oscillometry, followed by serum and exhaled breath condensate collection to estimate inflammatory and oxidative stress markers.

Impulse Oscillometry

The impulse oscillometry system (Eric Jaeger, Hoechberg, Germany) was used to measure the respiratory system impedance. It has two components, respiratory resistance and respiratory reactance at different frequencies. It is calculated from pressure and flow signals, where pressure is in phase with the flow.[29] Subjects were instructed to sit comfortably with their neck held in a neutral position. Measurement was performed for 60 seconds, during which subjects were asked to breathe normally after applying a nose clip, and their cheeks were supported firmly. The Loudspeaker near the mouthpiece delivered sound waves of different frequencies, ranging from 5 to 30 Hz, superimposed on spontaneous tidal breathing. Resistance and reactance measured at 5 Hz and 20 Hz frequency oscillations are designated R5, X5, R20, and X20, respectively. R5 gives total airway resistance as the sound waves of low frequency (5 Hz) are transmitted deep into the lungs up to the alveoli.

R20 provides central airway resistance. The difference between R5 and R20 (R5-R20) is considered an index of the small/peripheral airway resistance. Reactance has two components, inductance and capacitance. Inertance is the inertia of the air column and is positive, whereas capacitance reflects the elasticity of the lung and is negative in sign. The resonant frequency (Fres) is the intermediate frequency at which the total reactance is 0, and the area of reactance (Ax) is the integrated low-frequency respiratory reactance (area under the curve) between 5 Hz and Fres. It reflects a composite index for reactance. The correlation between airflow and pressure wave is known as coherence. Acceptable coherence values should be at least 0.8 or higher at 5 Hz and 0.9 or more at 20 Hz, demonstrating the reliability and quality of the given IOS test performance.[30-32]

Spirometry

Forced vital capacity (FVC) was measured according to the American Thoracic Society and European Respiratory Society guidelines using a spirometer (Medisoft, SpiroAir).[33,34]

Exhaled Breath Condensate (EBC) Collection

Exhaled breath condensate was collected using an R-tube (Respiratory Research, Inc., USA). R Tube is a disposable collection system that consists of a large Tee section made of polypropylene (PP), which separates saliva from the exhaled breath, a one-way valve (made of silicone rubber), and a PP collection tube, which is cooled by a cooling aluminium sleeve placed around it. Subjects were asked to perform tidal breathing, inhaling through the nose and exhaling into the mouthpiece connected to the R-tube for 10 minutes. Approximately 1.5 ml of the condensate was collected and immediately stored at -20 °C to estimate malondialdehyde (MDA), leukotriene B4, 8-isoprostane, and 3-nitrotyrosine.

Collection of Blood

Five milliliters of peripheral venous blood was collected under aseptic conditions from all subjects. Serum was separated and stored at -20 °C to estimate interleukin-6 (IL-6), IL-8, tumor necrosis factor α (TNF-α), and MDA.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed by using a 96-well microtiter plate pre-coated with a monoclonal antibody specific to the biomarker to be estimated.

Results

The demographic characteristics of 20 COPD patients and 25 healthy subjects are given in Table 1. The spirometric parameters in COPD patients and controls are shown in Table 2. FEV1 (% predicted), FVC (% predicted), and FEV1/FVC ratio were significantly lower in smokers-COPD and biomass smoke-exposed COPD patients compared to smokers and non-smoker controls. Multiple group comparison also shows that the smoker COPD patients had significantly less FEV1/FVC ratio than biomass smoke-exposed COPD patients.

Table 1. Demographic data of the study groups

Parameters	Smoker COPD (n=10)	Biomass COPD (n=10)	Smoker controls (n=10)	Non-smoker controls (n=15)	*P* value
Age (y)	60.27±5.89	54.40±7.04	59±8.74	56±10.24	0.375
Gender	M (10)	F(10)	M (10)	M (9), F (6)	-
Weight (kg)	60.55±6.36	58.80±5.95	58.71±5.85	61.20±6.30	0.690
Height (cm)	164.3±5.69	151.0±4.52	163.7±4.46	159.1±6.48	0.665
BMI (kg/m^2)	22.46±3.13	25.87±3.18	22.04±3.28	24.30±3.36	0.043

COPD: Chronic obstructive pulmonary disease, BMI: Body mass index.
Values are expressed as mean±standard deviation.

The IOS parameters for COPD patients and controls are given in Table 3. The resistance at 5 Hz, i.e., R5 (% predicted), was significantly higher in smoker COPD compared to smoker and non-smoker controls. However, no significant difference was observed between biomass-smoke-exposed and smoker COPD patients or between biomass-smoke-exposed COPD patients and controls. R20 (% predicted) showed no significant difference between COPD patients and controls. Reactance at 5 Hz (X5) was significantly less in smoker COPD than in control non-smokers. Reactance at 20 Hz, i.e., X20, was significantly lower or more negative in COPD patients than in the control group.

The parameters specific to peripheral airways (i.e., R5-R20, Fres, and Ax) were also significantly higher in COPD patients (smokers and biomass smoke exposed) than in healthy controls. Even though there is no significant difference between smoker COPD and biomass smoke-exposed COPD, there is a trend of more impairment of lung mechanics in smoker COPD as compared to biomass smoke-exposed COPD.

The systemic serum inflammatory biomarker analysis showed that serum IL-6 levels were significantly higher in smoker COPD patients than in non-smoker controls (as shown in Table 4). Among the oxidative stress markers, 8-isoprostane and nitrotyrosine-3 (NT3) levels in EBC were significantly higher in smoker COPD than in biomass smoke-exposed COPD and non-smoker controls,

Table 2. Intergroup comparison of spirometric parameters

Parameters	Smoker COPD (n = 10)	Biomass COPD (n = 10)	Smoker controls (n = 10)	Non-smoker controls (n = 15)	*P* value	Multiple comparison test
SVC (% predicted)	65.59 ± 17.68	62.21 ± 12.02	86.1 ± 16.06	93.93 ± 10.22	<.001***	0.010*† <0.001***‡‖ 0.04**¶
FEV_1 (% predicted)	39.83 ± 12.85	46.82 ± 11.18	84.93 ± 3.72	89.26 ± 9.21	<0.001***	<0.001***†‡¶‖
FVC (% predicted)	65.26 ± 22.18	62.24 ± 12.58	84.84 ± 8.54	92.94 ± 14.40	<0.001***	0.001**‡ 0.004*¶ 0.001**‖
FEV_1/FVC	50.89 ± 11.21	60.49 ± 6.53	75.27 ± 4.82	77.01 ± 6.86	<0.001***	0.040*§ <0.001***†‡‖ 0.005**¶

COPD: Chronic obstructive pulmonary disease, SVC: slow vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second.
§ Smoker COPD vs. Biomass COPD, † Smoker COPD vs. Smoker controls, ‡ Smoker COPD vs. Non-smoker controls, ¶ Biomass COPD vs. Smoker controls, ‖ Biomass COPD vs. Non-smoker controls.
Values expressed are mean ± standard deviation, analyzed by one-way ANOVA (post-hoc-Turkey).
*P value < 0.05, ** P value < 0.01 and *** P value < 0.001 statistically significant.

Table 3. Intergroup comparison of impulse oscillometry parameters

Parameters	Smoker COPD (n = 10)	Biomass COPD (n = 10)	Smoker controls (n = 10)	Non-smoker controls (n = 15)	*P* value	Multiple comparison test
R_5 (% predicted)	220.0 ± 55.49	198.8 ± 77.15	142.9 ± 31.24	134.5 ± 25.74	0.002**	0.028*† 0.003**‡
R_{20} (% predicted)	137.7 ± 32.04	134.3 ± 31.33	136.1 ± 24.63	123.2 ± 25.66	0.675	-
R_5-R_{20} [kPa/(L/S)]	0.33 (0.20-0.38)	0.35 (0.09-0.52)	0.06 (0.04-0.14)	0.08 (0.06-0.18)	0.001**	0.023*† 0.038*‡ 0.024*¶ 0.042*‖
X_5 [kPa/(L/S)]	-0.38 (-0.46-(-0.22))	-0.25 (-0.36-(-0.10))	-0.12 (-0.21-(-0.07))	-0.15 (-0.24-(-0.06))	0.013*	0.027*c
X_{20} [kPa/(L/S)]	-0.13 (-0.19-(-0.06))	-0.06 (-0.17-(-0.04))	0.06 (-0.02-0.06)	-0.005 (-0.04-0.05)	<0.001****	0.006**† 0.012*‡ 0.038*¶
Fres [1/S]	30.03 (23.14-33.52)	31.16 (26.36-39.59)	14.81 (13.02-19.08)	19.68 (14.93-25.56)	<0.001***	0.017*† 0.037*‡ 0.005**¶ 0.010**‖
Ax [kPa/L]	4.26 (2.37-5.14)	3.93 (1.50-5.41)	0.41 (0.27-1.49)	0.75 (0.30-2.12)	0.005**	0.049*† 0.039*‡

COPD: Chronic obstructive pulmonary disease, R_5: Resistance at 5 Hz; R_{20}: Resistance at 20 Hz; Fres: Resonant frequency; Ax: Area of reactance; X_5: Reactance at 5 Hz; X_{20}: Reactance at 20 Hz; R_5-R_{20}: Peripheral airway resistance.
§ Smoker COPD vs. Biomass COPD, † Smoker COPD vs. Smoker controls, ‡ Smoker COPD vs. Non-smoker controls, ¶ Biomass COPD vs. Smoker controls, ‖ Biomass COPD vs. Non-smoker controls.
Values expressed are mean ± standard deviation or median with inter-quartile range, analyzed by one-way ANOVA (post-hoc-Turkey) or Kruskal-Wallis test (post-hoc-Dunn's), respectively.
*P value < 0.05, ** P value < 0.01 and *** P value < 0.001 statistically significant.

Table 4. Intergroup comparison of serum and EBC biomarkers in COPD patients and controls

Parameters	Smoker COPD (n = 10)	Biomass COPD (n = 10)	Smoker controls (n = 10)	Non-smoker controls (n = 15)	*P* value	Multiple comparison tests
Serum TNF-α (pg/mL)	6.33 [2.86-16.22]	6.88 [4.43-16.16]	6.88 [5.49-9.76]	9.12 [3.82-22.22]	0.8488	-
Serum IL-6 (pg/mL)	10.26 [8.28-19.05]	11.31 [6.05-13.33]	7.14 [4.95-16.65]	6.11 [5.43-9.47]	0.0367*	0.0273*‡
Serum IL-8 (pg/mL)	31.16 ± 0.77	31.32 ± 0.83	30.52 ± 0.6	31.61 ± 1.66	0.4376	-
Serum MDA (nmol/mL)	7.63 [5.15-8.65]	6.75 [5.82-12.34]	7.32 [5.75-8.46]	8.19 [6.15-8.91]	0.888	-
EBC 8-Isoprostane (pg/mL)	29.44 ± 11.15	15.25 ± 6.65	5.10 ± 5.24	7.70 ± 7.74	< 0.0001***	0.03*§ < 0.0001***† < 0.0001***c
EBC NT3 (ng/mL)	25.94 [24.01-35.82]	22.37 [21.12-26.59]	20.61 [16.43-23.55]	17.48 [13.60-21.71]	0.0099**	0.0275*‡
EBC LT-B4 (ng/mL)	1.68 ± 0.20	1.42 ± 0.43	1.24 ± 0.26	1.29 ± 0.34	0.0554	-
EBC MDA (nmol/mL)	3.81 [2.57-4.32]	3.37 [2.91-6.74]	3.66 [2.87-4.23]	4.09 [3.08-4.45]	0.888	-

COPD: Chronic obstructive pulmonary disease, EBC: Exhaled breath condensate, TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6, IL-8: Interleukin-8, MDA: Malondialdehyde, NT-3: 3-Nitrotyrosine, LT-B4: Leukotriene B4.

§ Smoker COPD vs. Biomass COPD, † Smoker COPD vs. Smoker controls, ‡ Smoker COPD vs. Non-smoker controls.

Values expressed are mean ± standard deviation or median with inter-quartile range, analyzed by one-way ANOVA (post-hoc-Turkey) or Kruskal-Wallis test (post-hoc-Dunn's), respectively.

*P value < 0.05, ** P value < 0.01 and *** P value < 0.001 statistically significant.

respectively. In correlation analysis, R5 (% predicted) and FVC (%predicted) showed a positive correlation with serum IL-6 (P value: 0.013, r-value: 0.522) (P value: 0.022, r-value: 0.484), respectively, while R5-R20 positively correlated with serum NT3 (P value: 0.046, r-value: 0.451).

Discussion

This study measured lung volumes, capacities, respiratory, and impedance. We also estimated inflammatory and oxidative stress biomarkers in smoker-COPD and biomass smoke-exposed COPD patients to understand the characteristic changes in lung functions and inflammatory responses.

The lung function analysis showed significantly less FEV1/FVC in smoker-COPD. Except for FEV1/FVC, there are no significant differences in other lung volumes between biomass-smoke-exposed COPD and smoker-COPD. Both the COPD groups show a significant decrease in spirometry parameters as compared to control smokers and non-smokers. Several previous studies have also noted these findings.[35-40]

Respiratory impedance was measured using impulse oscillometry, which is much more precise and sensitive than spirometry in detecting the decline in lung function. Total airway resistance (R5) is significantly higher in smoker-COPD than in control groups. The parameters specific to dysfunction of peripheral airways, i.e., R5-R20, Fres, and Ax, were significantly higher in COPD patients (both smokers and biomass smoke exposed) compared to healthy controls, indicating involvement of peripheral airways and lung parenchyma in both types of COPD patients.

Reactance parameters, like X5 and X20, depict the rebound resistance produced by distensible airways. Reactance is mainly expressed in terms of inductance (L) and capacitance (C). At lower frequencies of 5 Hz, the capacitive properties of the peripheral airways dominate; there is a significant decrease in the reactance at 5 Hz in smoker-COPD compared with the smoker control. Several previous studies have also noted these changes.[41-47] In our study, even though impedance parameters are severely affected in smoker-COPD, we did not find any significant changes in IOS parameters between smoker-COPD and biomass smoke-exposed COPD, which is precisely against the findings of Salvi et al., where the non-smoker COPD (biomass exposed) lung mechanics parameters including resistance at 5 Hz, reactance curve and resonant frequency were significantly higher than that of smoker-COPD.[38]

We analyzed a set of biomarkers, including serum tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-8, MDA, and exhaled breath condensate 8-isoprostane, 3-nitrotyrosine (NT-3), leukotriene (LT)-B4, MDA. Our primary rationale was to identify the significant differences in levels of these biomarkers between smoker-COPD and biomass smoke-exposed COPD patients in serum and EBC samples.

COPD is characterized by predominant neutrophilic airway inflammation; cigarette smoke and other irritants mainly activate the alveolar macrophages and epithelial cells and cause release of different chemotactic mediators and cytokines, including IL-6, IL-8, IL-23, CXCL1, TNF-α, LT-B4, matrix metalloproteinases (MMPs) which attract the circulating immune cells like neutrophils, monocytes and lymphocytes to the lung tissue and further amplifies the inflammatory process.[18] Biomass smoke contains many components that are similar to those of tobacco smoke. It induces inflammatory responses by acting through transient potential receptor ion channels that are

present in the lung cells, and this leads to activation of downstream pathways resulting in the release of different inflammatory mediators like IL-6, IL-8, TNF-α, MMP, and monocyte chemoattractant proteins. Biomass smoke also initiates inflammatory pathways by activating toll-like receptors, further facilitating inflammasome formation and producing different inflammatory mediators.[48]

TNF-α is the most widely studied cytokine member of the TNF superfamily. It is mainly secreted by macrophages, T-cells, B-cells, and granulocytes.[49] We observed no significant differences in the levels of TNF-α within our study groups. Previous studies have also noted the same findings in COPD patients.[50-52] In contrast to our results, most of the past studies reported a significant increase in the levels of TNF-α in COPD patients.[53-56] T-cells and macrophages also secrete IL-6. It acts as a pro-inflammatory cytokine. We observed a significantly higher level of IL-6 in smoker COPD compared with non-smoker controls, which aligns with previous studies.[53,55,57-59] Even though biomass smoke-exposed COPD patients show higher levels of IL-6 than controls, the difference is insignificant.

IL-8 is also a chemokine released by the macrophages in the lungs, which induces the migration of neutrophils to the inflammatory site and orchestrates further reactions. We observed no significant difference in the IL-8 levels among the study groups. In contrast to our findings, previous studies noted a significant increase in the levels of IL-8.[53,60] LT-B4 is a major neutrophil chemotactic agent in the airways of COPD patients, mainly released by macrophages. Our study found no significant difference in the levels of LT-B4 in EBC samples within the study groups. However, there is a trend of increase in LT-B4 levels in smoker COPD compared to biomass smoke-exposed COPD and control groups.

Oxidative stress is also considered a major driving mechanism for COPD progression. In our study, we analyzed the serum levels of MDA and EBC levels of MDA, NT3, and 8-Isoprostane to investigate the oxidative stress status. Oxidative markers, including 8-Isoprostane and NT-3, showed a significant change in their levels compared to controls. 8-Isoprostane, a prostanoid mediator formed by free radical-catalyzed metabolism of arachidonic acid, was significantly higher in smoker-COPD compared to biomass smoke-exposed COPD and control groups. It shows that cigarette smoking causes severe disturbances in oxidative stress mechanisms, exacerbating the inflammatory process and resulting in severe lung function impairment. Biomass smoke-exposed COPD patients also show an increase in 8-isoprostane levels compared to controls, but it is not statistically significant.

Superoxide ions combine with NO to form peroxynitrite, mediating the formation of hydroxyl ions18. Peroxynitrite reacts with proteins to form NT3, a marker for nitrative stress and inflammation[61]. We observed significantly higher levels of NT3 in the EBC of smoker-COPD as compared to non-smoker controls. Our result is in accordance with a previous study done by Jin et al, where they reported that smoker-COPD has a significant increase in NT3 plasma protein levels compared to smokers without COPD.[61] Both cigarette smoking and biomass exposure produce ROS, cytokines, and lipid peroxidation products, which cause oxidative DNA damage or impair antioxidant mechanisms and further amplify the inflammatory process48. It shows that smoker-COPD patients have a decline in lung function due to inflammation, mainly driven by oxidative stress mechanisms.

Limitation

The most significant limitation of this study is its small sample size, resulting from the highly specific inclusion and exclusion criteria and the techniques employed.

Conclusion

This study reports a significant decline in lung functions, including pulmonary impedance, in both smoker-COPD and biomass smoke-exposed COPD patients as compared to both smoker controls and non-smoker controls. Smoker-COPD patients show higher levels of systemic inflammatory and airway oxidative stress markers.

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Authors' Contribution

Conceptualization: Geetanjali Bade, Anjana Talwar, Karan Madan, Animesh Ray.
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Writing–review & editing: Kumar Shanmugasundaram, Anjana Talwar, Karan Madan, Animesh Ray, Geetanjali Bade.

Competing Interests

No potential conflict of interest relevant to this article was reported.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Ethical Approval

The Institute ethics committee (AIIMS, New Delhi) approved the study protocol (Ref no. IEC-222/05.05.2017, RP-44/2017). Written informed consent was obtained from all the subjects before enrollment in the study.

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Study Highlights

What is current knowledge?

- Smoking is a major COPD risk factor, but biomass smoke also contributes substantially, especially in nonsmokers.
- COPD involves airway inflammation and oxidative stress; spirometry alone poorly detects smallairway disease.
- IOS and exhaled breath condensate (EBC) biomarkers offer noninvasive evaluation of airway function and inflammation.

What is new here?

- While both smoker- and biomass-smoke-exposed COPD similarly impair peripheral airway mechanics, smoker-COPD is distinguished by a more reduction in the FEV1/FVC ratio. The inflammatory markers in both serum and exhaled breath condensate are comparable with controls.

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