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Pulmonary Functions and Inflammatory Biomarkers in Post-Pulmonary Tuberculosis Sequelae

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Background: Post-tuberculosis (TB) sequelae is a commonly encountered clinical entity, especially in high TB burden countries. This may represent chronic anatomic sequelae of previously treated TB, with frequent symptomatic presentation. This pilot study was aimed to investigate the pulmonary functions and systemic inflammatory markers in patients with post-TB sequelae (PTBS) and to compare them with post-TB without sequelae (PTBWS) participants and healthy controls.

Methods: A total of 30 participants were enrolled, PTBS (n=10), PTBWS (n=10), and healthy controls (n=10). Pulmonary function tests included spirometry and measurement of airway impedance by impulse oscillometry. Serum levels of matrix metalloproteinase (MMP)-1, transforming growth factor-β, and interferon-γ were estimated.

Results: Slow vital capacity (SVC), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), FEV_1/FVC, and peak expiratory flow were significantly lower in PTBS as compared to controls. SVC and FEV_1 were significantly less in PTBS as compared to PTBWS. Total airway impedance (Z_5), total airway resistance (R_5), central airway resistance (R_{20}), area of reactance (Ax), and resonant frequency (Fres) were significantly higher and respiratory reactance at 5 and 20 Hz (X_5, X_{20}) were significantly lower in PTBS as compared to PTBWS. Spirometry parameters correlated with impulse oscillometry parameters in PTBS. Serum MMP-1 level was significantly higher in PTBS as compared to other groups.

Conclusion: Significant pulmonary function impairment was observed in PTBS, and raised serum MMP-1 levels compared with PTBWS and healthy controls. Follow-up pulmonary function testing is recommended after treatment of TB for early diagnosis and treatment of PTBS.

Keywords: Impulse Oscillometry; Inflammatory Markers; Post-TB Sequelae; Spirometry

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Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. Every year worldwide, around 10 million people are affected by TB. It is one of the top 10 causes of death[1]. About 85% of people who develop TB can be successfully treated with a 6-month first-line drug regimen. It is estimated that nearly half of the microbiologically cured pulmonary TB patients may develop post-TB sequelae (PTBS)[2].

Post TB sequelae is chronic anatomic and symptomatic (dyspnea/cough) sequelae from previously treated (microbiologically cured) pulmonary TB. Post-TB lung dysfunction often goes unrecognized, despite its relatively high prevalence and it is associated with reduced quality of life[3]. Pulmonary

dysfunction includes minor abnormalities to severe breathlessness with an increased risk of death[4]. Treated TB patients contribute to a growing worldwide burden of chronic obstructive pulmonary disease (COPD)[5]. Post TB patients are prone to develop a wide variety of non-infectious disorders; these include parenchymal disorders (thin-walled cavities, lung fibrosis), chronic airflow obstruction, bronchiectasis, subglottic and tracheobronchial stenosis, pleural thickening, corpulmonale, and chronic respiratory failure[6]. Chung et al.[7] found that the major nadir of pulmonary function impairment occurs approximately 18 months after completion of treatment. Several studies have reported that there is a decline in lung volumes and capacities that lead to obstructive and restrictive ventilatory defects during TB and also after completion of TB treatment[3,4,7]. Importantly, specific host and pathogen factors causing post TB lung impairment remain unclear. It is proposed that host immune responses may play a dominant role in lung damage, as excessive inflammation and elevated expression of lung matrix-degrading proteases are the hallmarks of TB pathogenesis. The inflammatory markers and cytokines that are released in response to active TB infection may cause severe damage and remodeling of the airways[8]. Matrix metalloproteinases (MMPs) are a family of 25 potent proteases that may degrade extracellular matrix components and are probably central to TB-associated lung injury[9]. Transforming growth factor-β (TGF-β), which is associated with lung inflammation, plays a crucial role in lung fibrosis[10]. Interferon-γ (IFN-γ) is also implicated in lung injury observed during TB[11].

No studies have specifically investigated airway impedance changes in patients diagnosed with post TB sequelae. The present pilot study aimed to assess lung functions using spirometry, impulse oscillometry and to estimate serum inflammatory biomarkers in patients with or without post TB sequelae.

Table 1. Demographic data of the study groups

Parameter	Control (n=10)	PTBS (n=10)	PTBWS (n=10)	p-value
Age, yr	37.50±9.95	46.20±10.05	37.10±9.52	0.083
Height, cm	166.50±14.19	158.40±12.63	167.90±4.47	0.146
Weight, kg	75.24±17.33	54.53±14.24	68.86±12.96	0.013*
BMI, kg/m^2	26.82±3.07	21.60±4.11	23.96±3.51	0.010*
Smoking history	-	-	-	-
Sex (male:female)	5:5	5:5	10:0	-

Values are expressed as mean±standard deviation, analyzed by one-way ANOVA.
*p<0.05 statistically significant.
PTBS: post-tuberculosis with sequelae; PTBWS: post-tuberculosis without sequelae; BMI: body mass index.

Table 2. Spirometry parameters of the study groups

Parameter	Control (n=10)	PTBS (n=10)	PTBWS (n=10)	p-value	Multiple comparisons test
SVC (% predicted)	86.20±17.20	60.60±18.28	79.21±10.12	0.003**	0.003**† 0.033*§
FVC (% predicted)	91.70±17.54	62.90±18.38	80.37±11.00	0.001**	0.001**†
FEV_1 (% predicted)	85.30±17.18	49.50±21.32	71.98±11.66	<0.001***	<0.001***† 0.018*§
FEV_1/FVC	78.05 (74.01–81.08)	63.21 (56.99–73.80)	77.88 (70.69–79.19)	0.048*	0.044*†
PEF (% predicted)	82.00±29.16	43.40±24.66	45.00±15.84	0.001**	0.004**† 0.003**‡

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<0.05, **p<0.01, and ***p<0.001 statistically significant. †Control vs. PTBWS. ‡Control vs. PTBS. §PTBWS vs. PTBS.
PTBS: post-tuberculosis with sequelae; PTBWS: post-tuberculosis without sequelae; SVC: slow vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; PEF: peak expiratory flow.

Materials and Methods

1. Study design

The study protocol was approved by the Institute Ethics Committee of All India Institute of Medical Sciences (AIIMS), New Delhi (Reference No: IECPG-791/31.01.2020). Enrolment of subjects was done as per the inclusion and exclusion criteria after obtaining written informed consent and willingness to participate in the study. The primary objectives of this pilot study were to compare lung functions and systemic inflammatory markers in patients with PTBS, post-TB without sequelae (PTBWS) and healthy controls. The study population included patients who had a history of microbiologically confirmed pulmonary TB, had completed anti-TB treatment and were declared bacteriologically cured. Based on a review of the clinical/clinico-radiological presentations, they were divided into two groups. Patients who had clinical symptoms and radiological evident abnormality on the chest radiograph were included in the PTBS group. Patients who had completed anti-TB treatment with no evidence of residual chest radiographic abnormality were included in the group PTBWS. Patients with a past/current history of smoking, asthma, COPD, sarcoidosis, interstitial lung diseases, and other respiratory diseases were excluded from this study. Also participants with active TB, history of multi-drug resistant TB, extrapulmonary TB, human immunodeficiency virus–TB, evidence of cardiovascular, musculoskeletal, chronic immunological diseases and inflammatory disorders were excluded from the study. A total of 30 participants were enrolled in this study with 10 participants in each group: PTBS (5 males and 5 females), PTBWS (10 males), and healthy controls (5 males and 5 females). Patient enrolment was carried out from the outpatient clinic at the Department of Pulmonary, Critical Care and Sleep Medicine, AIIMS, New Delhi. Age-matched healthy controls were also recruited. Assessment of lung functions and inflammatory markers was done at Respiratory Research Laboratory, Department of Physiology, AIIMS, New Delhi.

Table 3. Impulse oscillometry parameters of the study groups

Parameter	Control (n=10)	PTBS (n=10)	PTBWS (n=10)	p-value	Multiple comparisons test
Z_5	0.38±0.12	0.63±0.31	0.28±0.05	0.001**	0.020*† 0.001**§
Z_5 (% predicted)	117.70±28.53	195.30±100.80	102.30±20.16	0.004**	0.023*† 0.006**§
R_5	0.35±0.11	0.55±0.23	0.26±0.05	<0.001***	0.021*† <0.001***§
R_5 (% predicted)	111.50±27.57	171±73.66	97.59±19.15	0.003**	0.021*† 0.004**§
R_{20}	0.31±0.10	0.33±0.09	0.19±0.02	0.002**	0.012*† 0.002**§
R_{20} (% predicted)	93.49±26.91	120.40±34.43	83.99±9.96	0.011*	0.010*§
Ax	0.43±0.25	3.06±2.43	0.56±0.37	<0.001***	<0.001***† 0.001**§
Fres	14.39±3.76	28.75±7.87	18.63±3.21	<0.001****	<0.001****† <0.001***§
X_5	−0.09 (−0.18 to 0.07)	−0.22 (−0.45 to −0.10)	−0.07 (−0.08 to 0.06)	0.004**	0.003**§
X_5 (% predicted)	343.80 (−400 to 750)	1,163 (235.4 to 5,252)	−375 (−1,387 to 181)	0.015*	0.012*§
X_{20}	0.05±0.04	−0.07±0.07	0.003±0.01	<0.001****	<0.001****† 0.004**§
X_{20} (% predicted)	100 (27.68 to 111.10)	−113.30 (−267.50 to 16.94)	12.50 (−12.48 to 27.08)	0.001***	<0.001***†
R_5–R_{20}	0.05 (0.03 to 0.07)	0.13 (0.08 to 0.40)	0.08 (0.03 to 0.10)	0.009**	0.011*†
DeltaX_5	0.01 (0.01 to 0.02)	0.04 (0.02 to 0.09)	0.03 (0.01 to 0.05)	0.048*	0.048*†

Values presented 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<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 statistically significant. †Control vs. PTBWS. ‡Control vs. PTBS. §PTBWS vs. PTBS.
PTBS: post-tuberculosis with sequelae; PTBWS: post-tuberculosis without sequelae; Z_5: total airway impedance; R_5: resistance at 5 Hz; R_{20}: resistance at 20 Hz; Ax: area of reactance; Fres: resonant frequency; X_5: reactance at 5 Hz; X_{20}: reactance at 20 Hz; R_5–R_{20}: peripheral airway resistance.

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2. Data collection

History was taken regarding the duration of anti-TB treatment taken by the patients. All the recruited patients had completed anti-TB treatment and the treatment duration varied from 6 to 12 months. Baseline demographic data was recorded after recruitment.

3. Assessment of airway impedance by impulse oscillometry system

Assessment of airway impedance was done using the impulse oscillometry system (IOS; Eric Jaeger, Hochberg, Germany). Impulse oscillometry is a simple, non-invasive method to assess the mechanics of lungs and airways and it uses the

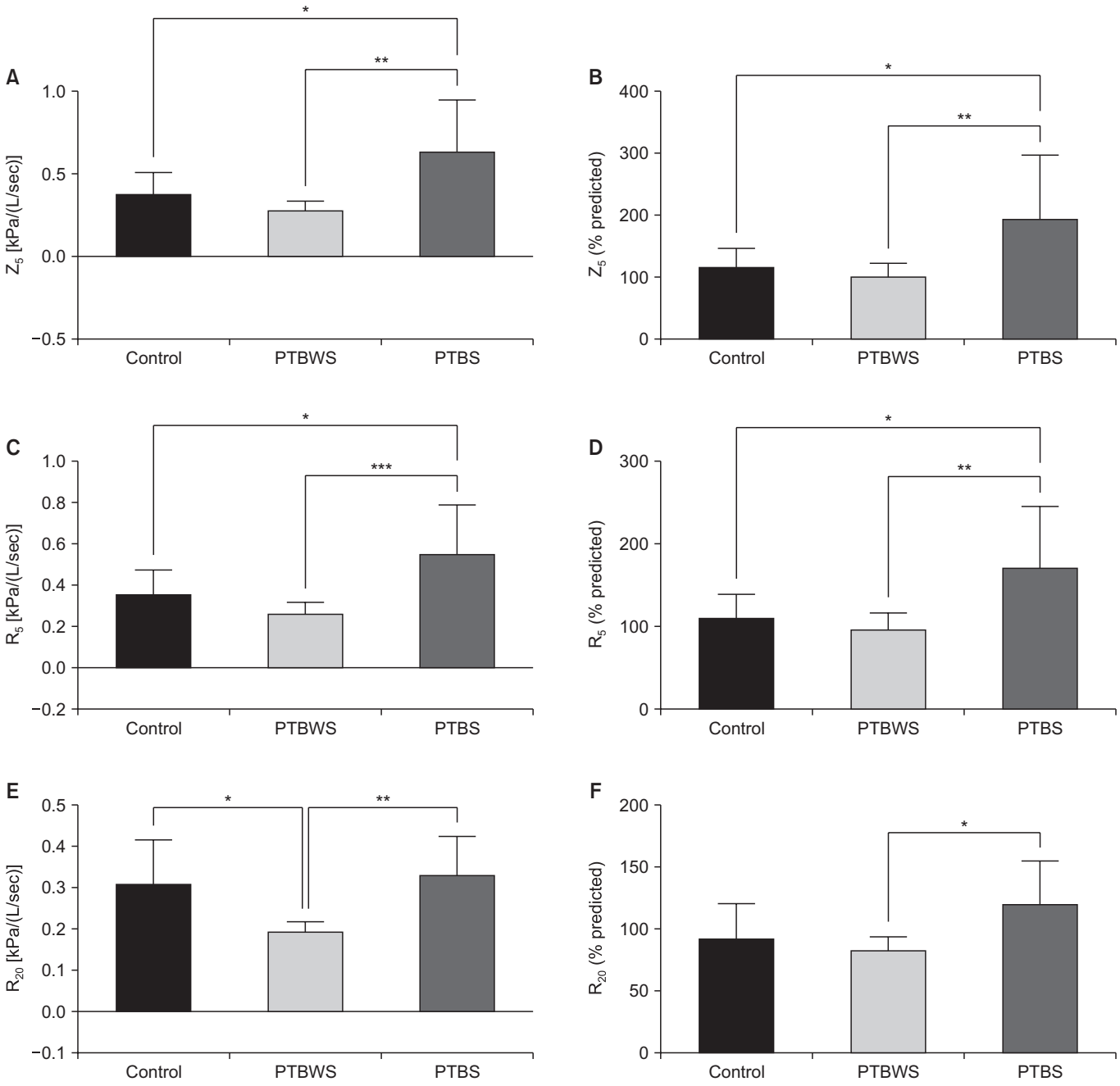

Figure 1. (A–F) Graph depicting the impulse oscillometry values of controls, post-tuberculosis without sequelae (PTBWS), and post-tuberculosis with sequelae (PTBS). Values are plotted as mean±standard deviation. *p<0.05, **p<0.01, and ***p<0.001 for intra group comparison. Z_5: total airway impedance; R_5: resistance at 5 Hz; R_{20}: resistance at 20 Hz.

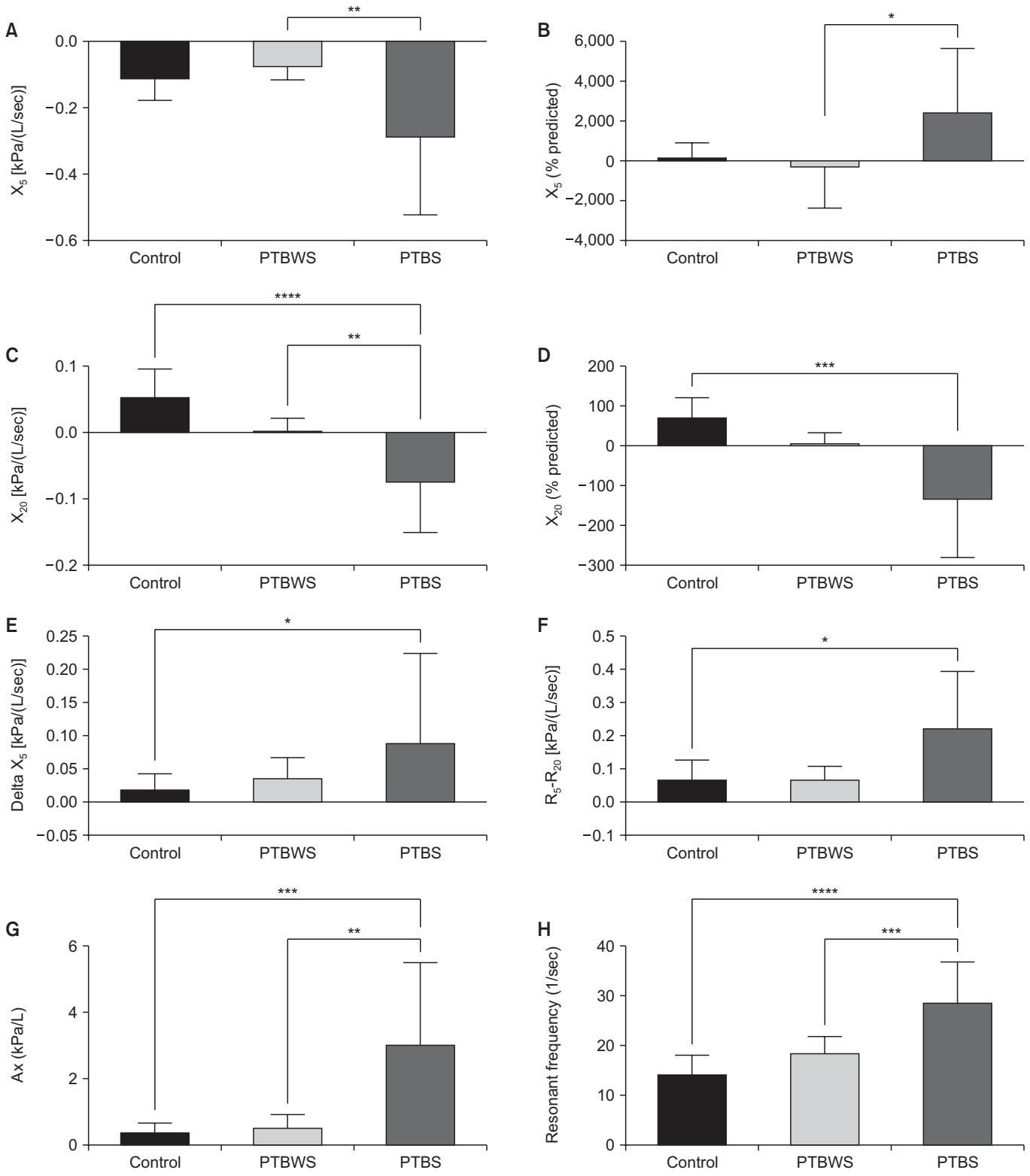

Figure 2. (A–H) Graph depicting the impulse oscillometry values of controls, post-tuberculosis without sequelae (PTBWS) and post-tuberculosis with sequelae (PTBS). Values are plotted as mean±standard deviation. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 for intra group comparison. X_5: reactance at 5 Hz; X_{20}: reactance at 20 Hz; R_5–R_{20}: peripheral airway resistance; Ax: area of reactance.

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forced oscillation technique. It requires minimal participant effort as compared to spirometry. Oscillating sound waves of different frequencies ranging between 5 Hz and 35 Hz are produced by loudspeaker and superimposed over normal tidal breathing. The lower frequencies travel deep into the lungs up to peripheral airways and reflected whereas the higher frequencies are reflected back from central airways. The test was performed in a sitting position for 90 seconds. A tight seal between lips and mouthpiece was ensured. The cheeks were held firmly by the patient with his/her hands. The parameters recorded were total airway impedance (Z_5), airway resistance at 5 and 20 Hz (R_5, R_{20}) and airway reactance at 5 and 20 Hz (X_5, X_{20}). The other oscillometry indices taken into consideration were peripheral airway resistance (R_5–R_{20}), resonant frequency (Fres), and area of reactance (Ax)[12,13].

4. Spirometry

The slow vital capacity (SVC) and forced vital capacity (FVC) maneuver were performed using the spirometer (Medisoft, Spiro Air, Kent, UK) and the parameters recorded were SVC, FVC, forced expiratory volume in 1 second (FEV_1), FEV_1/FVC ratio, and peak expiratory flow (PEF). The tests were performed as per the guidelines of the American Thoracic Society and European Respiratory Society[14].

5. Assessment of systemic inflammatory markers using enzyme-linked immunosorbent assay

Peripheral venous blood (3 mL) was collected under all aseptic precautions for the estimation of inflammatory markers: MMP-1, TGF-β, and IFN-γ. Serum was separated and stored at –20°C. Human enzyme-linked immunosorbent assay (ELISA) kits of Bioassay Technology Laboratory, China (cat Nos. E0916Hu, E0134Hu, E0105Hu) were used to quantify serum levels of MMP-1, TGF-β, and IFN-γ, respectively. ELISA was performed according to the manufactures guidelines and the color developed in the 96-well plates was read using a microplate reader (BioTek, Epoch 2 microplate reader, Winooski, VT, USA). Samples were estimated in duplicate and average values were used for analysis.

6. Statistical analysis

All statistical tests were done using GraphPad Prism version 9.0.1 for Windows (GraphPad Software, Inc., San Diego, CA, USA). Each parameter was tested for distribution of the data based on standard normality tests (D'Agostino-Pearson omnibus normality test, Anderson-Darling test, Shapiro-Wilk test). Multi-group comparisons were performed using one-way ANOVA or Kruskal-Wallis test with appropriate post hoc comparison test based on normality of data. The correlation between two parameters was evaluated using Pearson's correlation coefficient or Spearman's rank correlation coefficient

Table 4. Correlation between IOS with spirometry parameters of post-tuberculosis sequelae patients (n=10)

	SVC (% predicted)		FVC (% predicted)		FEV_1 (% predicted)		FEV_1/FVC		PEF (% predicted)	
	r value	p-value	r value	p-value	r value	p-value	r value	p-value	r value	p-value
R_5 (% predicted)†	–0.715	0.019*	–0.725	0.017*	–0.776	0.008**	–0.606	0.063	–0.719	0.019*
R_5†	–0.750	0.012*	–0.768	0.009**	–0.787	0.006**	–0.551	0.098	–0.726	0.017*
R_{20} (% predicted)‡	–0.477	0.163	–0.483	0.157	–0.470	0.170	–0.359	0.308	–0.555	0.095
R_{20}†	–0.511	0.130	–0.530	0.114	–0.489	0.151	–0.300	0.399	–0.582	0.077
X_5 (% predicted)‡	–0.565	0.093	–0.600	0.073	–0.636	0.054	–0.430	0.218	–0.697	0.030*
X_5†	0.704	0.022*	0.736	0.015*	0.720	0.018*	0.494	0.146	0.669	0.034*
X_{20} (% predicted)‡	0.695	0.030*	0.674	0.036*	0.784	0.009**	0.601	0.070	0.826	0.004**
X_{20}†	0.681	0.029*	0.677	0.031*	0.777	0.008**	0.602	0.065	0.661	0.037*
R_5–R_{20}‡	–0.737	0.018*	–0.741	0.017*	–0.863	0.002**	–0.644	0.049*	–0.820	0.005**
Ax†	–0.733	0.015*	–0.741	0.014*	–0.827	0.003**	–0.652	0.040*	–0.742	0.014*
Fres†	–0.426	0.219	–0.417	0.229	–0.591	0.071	–0.540	0.106	–0.548	0.100
Z_5 (% predicted)†	–0.695	0.025*	–0.716	0.019*	–0.747	0.013*	–0.566	0.088	–0.704	0.022*
Z_5†	–0.749	0.012*	–0.777	0.008**	–0.780	0.007**	–0.535	0.110	–0.728	0.016*

*p<0.05, **p<0.01 statistically significant. †Pearson correlation. ‡Spearman correlation.
IOS: impulse oscillometry system; SVC: slow vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; PEF: peak expiratory flow; R_5: resistance at 5 Hz; R_{20}: resistance at 20 Hz; X_5: reactance at 5 Hz; X_{20}: reactance at 20 Hz; R_5–R_{20}: peripheral airway resistance; Ax: area of reactance; Fres: resonant frequency; Z_5: total airway impedance.

if they were appropriate. Receiver operating characteristics (ROC) curve analysis was performed and likelihood ratio was used to determine the cutoff values of IOS parameters to distinguish between PTBS and PTBWS groups. The level of statistical significance was set at $p<0.05$.

Results

A total of 30 participants were enrolled in this pilot study with 10 participants in each group (PTBS, PTBWS, and healthy controls). The demographic data of these participants are presented in Table 1.

We observed that spirometry parameters: i.e., SVC (% predicted), FVC (% predicted), FEV_1 (% predicted), FEV_1/FVC, and PEF (% predicted) were significantly lower in PTBS as compared to healthy controls, while SVC (% predicted) and FEV_1 (% predicted) were significantly lower in PTBS as compared to PTBWS (Table 2).

Total airway impedance (Z_5), total airway resistance (R_5), peripheral airway resistance (R_5–R_{20}), area of reactance (Ax), and resonant frequency (Fres) were significantly higher and respiratory reactance at 20 Hz (X_{20}) were significantly lower in PTBS as compared to controls. In addition, central airway resistance (R_{20}) was significantly higher and reactance at 5 Hz (X_5) was significantly lower in PTBS as compared to PTBWS (Table 3, Figures 1, 2).

In PTBS patients, IOS parameters correlated with their spirometry parameters. There is a significant negative correlation between R_5, R_5 (% predicted), Z_5, Z_5 (% predicted), Ax, R_5–R_{20} with SVC (% predicted), FVC (% predicted), FEV_1 (% predicted), and PEF (% predicted). Likewise, R_5–R_{20} and Ax negatively correlated with all the spirometry parameters. The reactance parameters X_5 and X_{20} positively correlated with SVC (% predicted), FVC (% predicted), FEV_1 (% predicted), PEF (% predicted), and maximal expiratory flow (% predicted). R_{20}, R_{20} (% predicted), and Fres did not correlate with any of the spirometry parameters (Table 4). The correlation was also observed between IOS parameters and SVC, FVC in PTBWS subjects. As a significant difference was observed for IOS and spirometry parameters between PTBS and PTBWS, ROC curves were plotted to explore the ability of these parameters to discriminate between PTBS and PTBWS. The area under the curve (AUC), likelihood ratio, specificity, sensitivity, and their respective cutoff frequency to distinguish between the sequelae and without sequelae group are stated in Table 5. It was observed that all the parameters except FEV_1/FVC, PEF, and delta X_5 have AUC >0.8 and Z_5, R_5, and Ax are the most promising determining factors with AUC >0.9.

The median value of serum MMP-1 was significantly higher in PTBS (3.13 ng/mL) as compared to PTBWS (2.92 ng/mL). TGF-β levels were higher in PTBS (195.3 ng/L) as compared

Table 5. IOS a sensitive tool for lung function impairment in post TB sequelae

Parameter	Cutoff frequency	Sensitivity	Specificity	Likelihood ratio	AUC
Z_5	>0.355	0.9000	0.9000	9.00	0.9300
Z_5 (% predicted)	>131.5	0.6000	0.9000	6.00	0.8650
R_5	>0.325	0.9000	0.8000	4.50	0.9350
R_5 (% predicted)	>122.4	0.6000	0.9000	6.00	0.8500
R_{20}	> 0.215	0.8000	0.9000	8.00	0.8800
R_{20} (% predicted)	>95.55	0.8000	0.9000	8.00	0.8300
X_5	<–0.105	0.8000	0.9000	8.00	0.8950
X_5 (% predicted)	>1.312	0.5000	0.9000	5.00	0.8600
R_5–R_{20}	>0.110	0.6000	0.8000	3.00	0.8150
Fres	>22.44	0.8000	0.9000	8.00	0.8700
Ax	>0.955	0.8000	0.9000	8.00	0.9300
Delta X_5	>0.055	0.3000	0.9000	3.00	0.6100
SVC (% predicted)	<67.72	0.7000	0.9000	7.00	0.8200
FVC (% predicted)	<70.34	0.7000	0.9000	7.00	0.8100
FEV_1 (% predicted)	<60.22	0.7000	0.9000	7.00	0.8300
FEV_1/FVC	<67.17	0.6000	0.9000	6.00	0.7000
PEF (% predicted)	<28.67	0.4000	0.9000	4.00	0.5700

IOS: impulse oscillometry system; TB: tuberculosis; AUC: area under the curve; Z_5: total airway impedance; R_5: resistance at 5 Hz; R_{20}: resistance at 20 Hz; X_5: reactance at 5 Hz; R_5–R_{20}: peripheral airway resistance; Fres: resonant frequency; Ax: area of reactance; SVC: slow vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; PEF: peak expiratory flow.

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Table 6. Levels of inflammatory markers in the study groups

	Control (n=10)	PTBS (n=10)	PTBWS (n=10)	p-value
MMP-1 (ng/mL)	2.928 (1.136–5.366)	3.134 (1.847–3.600)	1.115 (0.866–1.915)	0.020*
TGF-β (ng/L)	173.7 (120.2–264.5)	195.3 (147.5–212.6)	141.1 (111.8–150.5)	0.100
INF-γ (ng/mL)	20.62 (17.99–25.80)	14.20 (12.27–17.98)	15.59 (10.22–18.65)	0.102

Values expressed are median with inter-quartile range, analyzed by Kruskal-Wallis test.
*p<0.05 statistically significant.
PTBS: post-tuberculosis with sequelae; PTBWS: post-tuberculosis without sequelae; MMP-1: matrix metalloproteinase-1; TGF-β: transforming growth factor β; INF-γ: interferon γ.

with PTBWS (141.1 ng/L) but the difference was statistically insignificant and both the data were comparable with healthy controls (Table 6). Serum IFN-γ levels are comparable within the study groups. A statistically significant positive correlation was observed between the serum levels of MMP-1 and TGF-β in PTBS (r=0.785, p=0.027).

Discussion

In the present study, we have measured the airway impedance and spirometry parameters in patients diagnosed with post TB sequelae. To the best of our knowledge, this is the first study investigating the airway impedance in post-TB patients using IOS. We observed significantly lower lung volumes and capacities in PTBS patients as compared with PTBWS and healthy controls. Most of the parameters like SVC, FVC, FEV_1, FEV_1/FVC ratio, and PEF were reduced in sequelae patients. Out of 10 patients, nine patients had mixed restrictive and obstructive respiratory impairment and one had normal lung function. Previous studies also indicate that there is an impaired lung function in patients who had sequelae at the end of TB treatment. The radiological signs of these patients were correlated with spirometry parameters[4,15,16].

The total airway impedance (Z_5) is the sum of all resistive, inertial, and elastic forces of the respiratory system, the sound waves have to encounter during their travel through the respiratory system. The significant increase in total airway impedance (Z_5) (Z_5 % predicted) in PTBS patients shows that there is impaired lung mechanics in these patients. Resistance shows the amount of resistance offered to the flow by the airways. We observed higher total airway resistance (R_5) (R_5 % predicted), central airway resistance (R_{20}) (R_{20} % predicted) and peripheral airway resistance (R_5–R_{20}) in PTBS as compared to PTBWS and controls.

Reactance is the rebound resistance produced by distensible airways. It includes the mass-inertial forces of the moving air column expressed in terms of inertance (I) and the elastic properties or compliance of the lung periphery expressed as capacitance (C). At lower frequencies, i.e., 5 Hz, capacitative properties of the small peripheral airways dominate. In this study, we have observed X_5 significantly lower (more negative) in PTBS as compared to PTBWS. Reduced elasticity of the lungs, due to the presence of fibrosis and hyperinflation can make the capacitance increasingly negative[12]. Thus lower X_5 may suggest the presence of disturbed physical properties of the lung parenchyma and its inability to expand and facilitate alveolar filling in the PTBS patient group. We also found that the area of reactance (Ax) and resonant frequency (Fres) in PTBS patients were higher as compared with other study groups. Ax, Fres, and X_5 act as sensitive parameters to determine the small airway obstruction and restrictive airway diseases[17]. The change in all of these IOS and spirometric parameters show that PTBS patients have significant impairment in the airway mechanics and have combined airway obstruction and restriction. This impairment in airway mechanics is may be due to remodeling of lung tissue observed during TB infection and its recovery. The release of different inflammatory mediators like MMP-1 and TGF-β during TB destroy the peripheral lung extracellular matrix and lead to pulmonary fibrosis respectively[15,16].

We have also studied the correlation between spirometry and IOS parameters in PTBS and observed that SVC (% predicted), FVC (% predicted), FEV_1 (% predicted) and PEF (% predicted) correlate negatively with Z_5, Z_5 (% predicted), R_5, R_5 (% predicted), Ax and R_5–R_{20} and positively with X_5 and X_{20}. This shows that a decrease in lung volumes and capacities is associated with an increase in airway resistance and a decrease in airway reactance. The increase in airway resistance is mainly caused due to damage and remodeling of peripheral airways during the TB infection, course of treatment and post-treatment depending upon the pathogen-host interaction. Our results are in agreement with the study conducted by Xia Wei et al., where they observed a correlation between spirometry parameters FEV_1 (% predicted), maximal (mid-) expiratory flow 75%–25%, and residual volume/total lung capacity and IOS parameters Z_5 (% predicted), R_5, R_{20}, R_5–R_{20}% R_5, R_5, R_5% predicted, Fres, Ax, X_5, and also reported that IOS can be an alternative diagnostic method for COPD[18]. ROC curve analysis shows that IOS parameters like Z_5, R_5, R_{20}, X_5, Ax, and Fres act as the most sensitive parameter to differentiate PTBS from PTBWS. There was no correlation found between IOS parameters and inflammatory biomarkers in PTBS.

We observed significantly higher serum levels of MMP-1

in PTBS patients as compared with PTBWS. Serum MMP-1 is one of the proteases in the family of 25 potent proteases of MMPs that usually degrade extracellular matrix components and play a key role in TB-associated lung injury. Studies suggest that there is an increase in the levels of MMP-1 and MMP-9 gene expression that is associated with damage to lung parenchyma during TB[19]. We also observed that MMP-1 levels in both post TB groups were comparable to healthy controls. It is observed that levels of MMP-1 significantly decrease during the course of treatment[20] and the first-line antimycobacterial agents specifically, moxifloxacin suppress MMP-1 secretion and gene expression in human airway epithelial cells[21]. Another inflammatory biomarker we assessed was TGF-β, found to be the principal mediator of pulmonary fibrogenesis[22]. It stimulates differentiation of fibroblasts into myofibroblasts that then produce α-smooth muscle actin, a key indicator and contributor to fibrotic pathogenesis. In our study, we found that the serum levels of TGF-β were comparable within the study groups. But, there is a trend of increased TGF-β levels observed in PTBS patients as compared with PTBWS. Christine et al.[10], have reported similar findings just after the completion of TB treatment. A positive correlation was observed between MMP-1 and TGF-β levels of PTBS; it indicates that increased levels of these inflammatory markers may simultaneously play a role in the remodeling of the airways during the course of the disease process and its treatment. IFN-γ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. IFN-γ released by $CD4^+$ T cells of the TH1 subset act as an important activator of macrophages. This leads to the release of more inflammatory mediators by macrophages and the recruitment of more and more inflammatory cells that form the granuloma during TB[23-25]. In the present study, the serum levels of IFN-γ were comparable within all the study groups. It has also been reported that IFN-γ levels are increased during TB and decrease at the end of anti-TB treatment[26-28].

Post-pulmonary TB sequelae is an emerging worldwide burden of lung function impairment after a complete course of TB treatment. It is likely that specific host-pathogen interactions occur during TB treatment. There is an urgent need to investigate the profile of inflammatory markers and host-pathogen interaction during the course of TB treatment and post-treatment follow-up for a prolonged period to understand the exact pathophysiological basis of PTBS. It may facilitate early detection of post TB sequelae, optimization of the treatment methods and improvement in the quality of life of post-TB patients.

PTBS patients have reduced lung volumes and capacities along with impaired lung mechanics. In these patients, spirometry parameters correlated significantly with impulse oscillometry parameters. Significantly higher serum MMP-1 level is observed in post TB with sequelae as compared to PTBWS subjects.

This study provides the importance of doing follow-up spirometry and impulse oscillometry in patients diagnosed with pulmonary TB after completing the anti-TB treatment. It will help in early diagnosis of pulmonary impairment.

To the best of our knowledge, this is the first of its kind study in which lung volumes, capacities and airway mechanics are studied in patients having post-pulmonary TB sequelae and compared with post-pulmonary TB without sequelae participants and healthy controls. The most important limitations of this study are its small sample size and less number of estimated serum cytokines. But with this small sample size also we observed significant impairment in lung volumes, capacities and airway mechanics in patients having post-pulmonary TB sequelae. Further to understand pathophysiology of post-pulmonary TB sequelae, it is important to do a follow-up study in patients during and after completion of anti-TB treatment with large sample size and whole profile of biomarkers must be estimated.

Author's Contributions

Conceptualization: Bade G, Talwar A, Madan K. Formal analysis: Shanmugasundaram K. Data curation: Shanmugasundaram K. Writing - original draft preparation: Shanmugasundaram K. Writing - review and editing: Shanmugasundaram K, Talwar A, Madan K, Bade G. Approval of final manuscript: all authors.

Conflicts of Interest

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

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