Title: Pulmonary functions and inflammatory biomarkers in Post Pulmonary Tuberculosis Sequelae.

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Running Head Pulmonary impairment after tuberculosis
Author’s Contributions

Concept and intellectual content were proposed by Geetanjali Bade, Anjana Talwar and Karan Madan. Patient recruitment, data collection, analysis and manuscript preparation were performed by Shanmugasundaram. All the authors read and approved the manuscript.
Abstract

Background: Post-tuberculosis sequelae is a commonly encountered clinical entity, especially in high tuberculosis (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-beta (TGF-β) and interferon-gamma (INF-γ) were estimated.

Results: Slow vital capacity (SVC), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC and peak expiratory flow (PEF) were significantly lower in PTBS as compared to controls. SVC and FEV1 were significantly less in PTBS as compared to PTBWS. Total airway impedance (Z5), total airway resistance (R5), central airway resistance (R20), area of reactance (Ax) and resonant frequency (Fres) were significantly higher and respiratory reactance at 5 and 20Hz (X5, X20) 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 (PTBS), Spirometry.
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-months first-line drug regimen. It is estimated that nearly half of the microbiologically cured pulmonary TB patients may develop post TB sequelae\(^2\).

Post TB sequelae is chronic anatomic and symptomatic (dyspnea/cough) sequelae from previously treated (microbiologically cured) pulmonary tuberculosis. 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, cor pulmonale and chronic respiratory failure\(^6\). Chung et al. found that the major nadir of pulmonary function impairment occurs approximately 18 months after completion of treatment\(^7\). 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. Transforming growth factor-beta (TGF-β), which is associated with lung inflammation, plays a crucial role in lung fibrosis. Interferon-gamma (INF-γ) is also implicated in lung injury observed during TB.

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.

Materials and methods

Study design

The study protocol was approved by the Institute Ethics Committee of 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 post-TB sequelae, post-TB without sequelae and healthy controls. The study population included patients who had a history of microbiologically confirmed pulmonary TB, had completed anti-tuberculosis 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 post-TB sequelae group (PTBS). Patients who had completed anti-TB treatment with no evidence of residual chest radiographic abnormality were included in the group post TB without sequelae (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, extra-pulmonary TB, human immunodeficiency virus (HIV)-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.

**Data collection**

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

**Assessment of airway impedance by Impulse Oscillometry system (IOS)**

Assessment of airway impedance was done using the Impulse Oscillometry System (Eric Jaeger, Hochberg, Germany). Impulse Oscillometry is a simple, non-invasive method to assess the mechanics of lungs and airways and it uses the forced oscillation technique. It requires minimal participant effort as compared to spirometry. Oscillating sound waves of different frequencies ranging between 5Hz and 35Hz 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 ($F_{res}$) and area of reactance ($A_x$).12,13

**Spirometry**

The slow vital capacity and forced vital capacity manoeuvre were performed using the spirometer (Medisoft, Spiro Air, Kent, UK) and the parameters recorded were slow vital capacity ($SVC$), forced vital capacity ($FVC$), 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

**Assessment of systemic inflammatory markers using enzyme-linked immunosorbent assay (ELISA).**

Peripheral venous blood (3ml) was collected under all aseptic precautions for the estimation of inflammatory markers- matrix metalloproteinase (MMP) -1, transforming growth factor-beta (TGF-$\beta$) and interferon-gamma (INF $\gamma$). Serum was separated and stored at -20°C. Human ELISA kits of Bioassay technology laboratory, China (Cat No: E0916Hu, E0134Hu, E0105Hu) were used to quantify serum levels of MMP-1, TGF-$\beta$ and INF-$\gamma$ 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, USA). Samples were estimated in duplicate and average values were used for analysis.

**Statistical analysis**

All statistical tests were done using GraphPad Prism version 9.0.1 for Windows (GraphPad Software, Inc., 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 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), FEV₁ (% predicted), FEV₁/FVC and PEF (% predicted) were significantly lower in PTBS as compared to healthy controls, while SVC (% predicted) and FEV₁ (% predicted) were significantly lower in PTBS as compared to PTBWS (Table 2).

Total airway impedance (Z₅), total airway resistance (R₅), peripheral airway resistance (R₅-R₂₀), area of reactance (Ax) and resonant frequency (Fres) were significantly higher and respiratory reactance at 20Hz (X₂₀) were significantly lower in PTBS as compared to controls. In addition, central airway resistance (R₂₀) was significantly higher and reactance at 5Hz (X₅) was significantly lower in PTBS as compared to PTBWS (Table 3).

In PTBS patients, IOS parameters correlated with their spirometry parameters. There is a significant negative correlation between R₅, R₅ (% predicted), Z₅, Z₅ (% predicted), Ax, R₅-R₂₀ with SVC (% predicted), FVC (% predicted), FEV₁ (% predicted), and PEF (% predicted). Likewise, R₅-R₂₀ 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 MEF(% predicted). $R_{20}$, $R_{20}$ (% predicted) and $F_{res}$ 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 cut-off 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 $A_x$ are the most promising determining factors with AUC>0.9.

**Inflammatory biomarkers**

The median value of serum MMP-1 was significantly higher in PTBS (3.13ng/ml) as compared to PTBWS (2.92ng/ml). TGF-$\beta$ levels were higher in PTBS (195.3ng/L) as compared with PTBWS (141.1ng/L) but the difference was statistically insignificant and both the data were comparable with healthy controls (Table 6). Serum INF-$\gamma$ levels are comparable within the study groups. A statistically significant positive correlation was observed between the serum levels of MMP-1 and TGF-$\beta$ in PTBS (r-value: 0.785) (p-value <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, 9 patients had mixed restrictive and
obstructive respiratory impairment and 1 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 \(Z_5\) \(Z_5 \% \text{ predicted}\) 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 \(R_5\) \(R_5 \% \text{ predicted}\), central airway resistance \(R_{20}\) \(R_{20} \% \text{ predicted}\) 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
\(A_x\) and resonant frequency \(F_{res}\) in PTBS patients were higher as compared with other
study groups. \(A_x\), \(F_{res}\) 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\textsuperscript{15,16}.

We have also studied the correlation between spirometry and IOS parameters in PTBS and
observed that SVC (% predicted), FVC (% predicted), FEV\textsubscript{1} (% predicted) and PEF (%
predicted) correlate negatively with Z\textsubscript{5}, Z\textsubscript{5} (% predicted), R\textsubscript{5}, R\textsubscript{5} (% predicted), Ax and R\textsubscript{5}-R\textsubscript{20}
and positively with X\textsubscript{5} and X\textsubscript{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\textsubscript{1} (% predicted),
MMEF 75\%–25\%,and residual volume/total lung capacity and IOS parameters Z\textsubscript{5} (%
predicted), R\textsubscript{5}, R\textsubscript{20}, R\textsubscript{5}-R\textsubscript{20} % R\textsubscript{5}, R\textsubscript{5}, R\textsubscript{5} % predicted, Fres, Ax, X\textsubscript{5}, and also reported that IOS
can be an alternative diagnostic method for chronic obstructive pulmonary disease\textsuperscript{18}. ROC
curve analysis shows that IOS parameters like Z\textsubscript{5}, R\textsubscript{5}, R\textsubscript{20}, X\textsubscript{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 matrix
metalloproteinases 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-
land MMP- 9 gene expression that is associated with damage to lung parenchyma during
TB\textsuperscript{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 treatment²⁰ and the first-line antituberculosis agents specifically, moxifloxacin suppress MMP-1 secretion and gene expression in human airway epithelial cells²¹. Another inflammatory biomarker we assessed was TGF-β, found to be the principal mediator of pulmonary fibrogenesis²². It stimulates differentiation of fibroblasts into myofibroblasts that then produce alpha-smooth muscle actin (αSMA), 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., have reported similar findings just after the completion of TB treatment¹⁰. 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 TB²³–²⁵. 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 tuberculosis and decrease at the end of anti-tuberculosis treatment²⁶–²⁸.

Post pulmonary tuberculosis 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 post-TB sequelae. 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.

**Conclusion**

Post tuberculosis with sequelae 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 post TB without sequelae subjects.

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

**Limitation**

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.

**Conflicts of interest**

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

**Acknowledgement**
We acknowledge all the participants who took part in this study.

Funding

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Table 1. Demographic data of the study groups

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls(n=10)</th>
<th>PTBS(n=10)</th>
<th>PTBWS(n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>37.50 ± 9.95</td>
<td>46.20±10.05</td>
<td>37.10 ±9.52</td>
<td>0.0835</td>
</tr>
<tr>
<td>Height (Cm)</td>
<td>166.5 ± 14.19</td>
<td>158.4±12.63</td>
<td>167.9 ±4.47</td>
<td>0.1464</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>75.24 ± 17.33</td>
<td>54.53±14.24</td>
<td>68.86 ±12.96</td>
<td>0.0138*</td>
</tr>
<tr>
<td>BMI(Kg/m²)</td>
<td>26.82 ± 3.07</td>
<td>21.60 ± 4.11</td>
<td>23.96 ±3.51</td>
<td>0.0107*</td>
</tr>
<tr>
<td>Smoking history</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Males(n=5)</td>
<td>Males(n=5)</td>
<td>Males(n=10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females(n=5)</td>
<td>Females(n=5)</td>
<td></td>
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</tr>
</tbody>
</table>

Table 2. Spirometry parameters of the study groups

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, † 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; FEV1: forced expiratory volume in 1 second; PEF: peak expiratory flow.
Table 3. Impulse oscillometry parameters of the study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=10)</th>
<th>PTBS (n=10)</th>
<th>PTBWS (n=10)</th>
<th>p-value</th>
<th>Multiple comparisons test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z₅</td>
<td>0.381 ± 0.125</td>
<td>0.634 ± 0.311</td>
<td>0.2810 ± 0.054</td>
<td>0.0013**</td>
<td>0.0204***† ‡ 0.0012***§</td>
</tr>
<tr>
<td></td>
<td>(% predicted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z₄</td>
<td>117.7 ± 28.53</td>
<td>195.3 ± 100.8</td>
<td>102.3 ± 20.16</td>
<td>0.0048**</td>
<td>0.0234***† ‡ 0.0061***§</td>
</tr>
<tr>
<td>R₅</td>
<td>0.359 ± 0.116</td>
<td>0.556 ± 0.234</td>
<td>0.2680 ± 0.0511</td>
<td>0.0009***</td>
<td>0.0214***† ‡ 0.0008***§</td>
</tr>
<tr>
<td></td>
<td>(% predicted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₅</td>
<td>111.5 ± 27.57</td>
<td>171.0 ± 73.66</td>
<td>97.59 ± 19.15</td>
<td>0.0036**</td>
<td>0.0218***† ‡ 0.0043***§</td>
</tr>
<tr>
<td>R₂₀</td>
<td>0.310 ± 0.105</td>
<td>0.332 ± 0.091</td>
<td>0.1970 ± 0.0211</td>
<td>0.0020**</td>
<td>0.0123***† ‡ 0.0027***§</td>
</tr>
<tr>
<td>R₃₀</td>
<td>93.49 ± 26.91</td>
<td>120.4 ± 34.43</td>
<td>83.99 ± 9.964</td>
<td>0.0113*</td>
<td>0.0109**</td>
</tr>
<tr>
<td>Ax</td>
<td>0.4350 ± 0.2513</td>
<td>3.063 ± 2.436</td>
<td>0.568 ± 0.377</td>
<td>0.0004***</td>
<td>0.0009***† ‡ 0.0016***§</td>
</tr>
<tr>
<td>Fres</td>
<td>14.39 ± 3.766</td>
<td>28.75 ± 7.877</td>
<td>18.63 ± 3.217</td>
<td>&lt; 0.0001****</td>
<td>&lt;0.0001****† ‡ 0.0007***§</td>
</tr>
<tr>
<td>X₅</td>
<td>-0.09[-0.18, -0.07]</td>
<td>-0.22[-0.45,-0.10]</td>
<td>-0.07 [-0.08,-0.06]</td>
<td>0.0046**</td>
<td>0.0034**†</td>
</tr>
<tr>
<td></td>
<td>(-%400, -750.0]</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>X₅</td>
<td>343.8 [-200, 750.0]</td>
<td>1163 [235.4 - 5252]</td>
<td>-375[-1387, -181]</td>
<td>0.0156*</td>
<td>0.0127**†</td>
</tr>
<tr>
<td></td>
<td>(% predicted)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>X₂₀</td>
<td>0.054 ± 0.041</td>
<td>-0.075 ± 0.074</td>
<td>0.003 ± 0.018</td>
<td>&lt; 0.0001****</td>
<td>&lt;0.0001****† ‡ 0.0049***§</td>
</tr>
<tr>
<td></td>
<td>(% predicted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₂₀</td>
<td>100 [27.68, 111.1]</td>
<td>-113.3[(-267.5), (16.94)]</td>
<td>12.5[(-12.48), -27.08]</td>
<td>0.0002***</td>
<td>0.0001***†</td>
</tr>
<tr>
<td>R₅-R₂₀</td>
<td>0.05 [0.03, 0.07]</td>
<td>0.13 [0.08, 0.40]</td>
<td>0.08 [0.03, 0.10]</td>
<td>0.0092**</td>
<td>0.0110†</td>
</tr>
<tr>
<td>DeltaX₅</td>
<td>0.01 [0.01, 0.02]</td>
<td>0.04 [0.02, 0.09]</td>
<td>0.03 [0.01, 0.05]</td>
<td>0.0484*</td>
<td>0.0483**</td>
</tr>
</tbody>
</table>

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, ***p-value <0.001 and ****p-value <0.0001 statistically significant, †control vs PTBWS; ‡control vs PTBS; §PTBWS vs PTBS.
PTBS: post tuberculosis with sequelae; PTBWS: post tuberculosis without sequelae; Z₅: total airway impedance; R₅: resistance at 5Hz; R₂₀: resistance at 20Hz; Fres: resonant frequency; Ax: area of reactance; X₅: reactance at 5Hz; X₂₀: reactance at 20Hz; R₅-R₂₀: peripheral airway resistance.
Table 4. Correlation between IOS with spirometry parameters of post tuberculosis sequelae patients (n=10).

<table>
<thead>
<tr>
<th></th>
<th>SVC (% predicted)</th>
<th>FVC (% predicted)</th>
<th>FEV₁ (% predicted)</th>
<th>FEV₁/FVC</th>
<th>PEF (% predicted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r value</td>
<td>p-value</td>
<td>r value</td>
<td>p-value</td>
<td>r value</td>
<td>p-value</td>
</tr>
<tr>
<td>‡ R₅ (% predicted)</td>
<td>-0.715</td>
<td>0.019*</td>
<td>-0.725</td>
<td>0.017*</td>
<td>-0.776</td>
</tr>
<tr>
<td>‡ R₅</td>
<td>-0.750</td>
<td>0.012*</td>
<td>-0.768</td>
<td>0.0093**</td>
<td>-0.787</td>
</tr>
<tr>
<td>‡ R₂₀ (% predicted)</td>
<td>-0.477</td>
<td>0.1633</td>
<td>-0.483</td>
<td>0.1570</td>
<td>-0.470</td>
</tr>
<tr>
<td>‡ R₂₀</td>
<td>-0.511</td>
<td>0.1306</td>
<td>-0.530</td>
<td>0.1149</td>
<td>-0.489</td>
</tr>
<tr>
<td>‡ X₅ (% predicted)</td>
<td>-0.565</td>
<td>0.093</td>
<td>-0.600</td>
<td>0.073</td>
<td>-0.636</td>
</tr>
<tr>
<td>‡ X₅</td>
<td>0.7048</td>
<td>0.0228*</td>
<td>0.7369</td>
<td>0.0150*</td>
<td>0.7207</td>
</tr>
<tr>
<td>‡ X₂₀ (% predicted)</td>
<td>0.695</td>
<td>0.030*</td>
<td>0.674</td>
<td>0.036*</td>
<td>0.784</td>
</tr>
<tr>
<td>‡ X₂₀</td>
<td>0.6818</td>
<td>0.0299*</td>
<td>0.6775</td>
<td>0.0314*</td>
<td>0.7772</td>
</tr>
<tr>
<td>‡ R₅-R₂₀ (% predicted)</td>
<td>-0.737</td>
<td>0.018*</td>
<td>-0.741</td>
<td>0.017*</td>
<td>-0.863</td>
</tr>
<tr>
<td>‡ Ax</td>
<td>-0.733</td>
<td>0.015*</td>
<td>-0.741</td>
<td>0.014*</td>
<td>-0.827</td>
</tr>
<tr>
<td>‡ Fres</td>
<td>-0.426</td>
<td>0.219</td>
<td>-0.417</td>
<td>0.229</td>
<td>-0.591</td>
</tr>
<tr>
<td>‡ Z₅ (% predicted)</td>
<td>-0.695</td>
<td>0.025*</td>
<td>-0.716</td>
<td>0.019*</td>
<td>-0.747</td>
</tr>
<tr>
<td>‡ Z₅</td>
<td>-0.749</td>
<td>0.0126*</td>
<td>-0.777</td>
<td>0.0081**</td>
<td>-0.780</td>
</tr>
</tbody>
</table>

*p-value<0.05, **p-value<0.01 statistically significant, ‡pearson correlation, ‡spearman correlation.

Z₅: total airway impedance; R₅: resistance at 5Hz; R₂₀: resistance at 20Hz; Fres: resonant frequency; Ax: area of reactance; X₅: reactance at 5Hz; X₂₀: reactance at 20Hz; R₅-R₂₀: peripheral airway resistance; SVC: slow vital capacity; FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 second; PEF: peak expiratory flow.
Table 5. IOS a sensitive tool for lung function impairment in post TB sequelae.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cut-off frequency</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Likelihood ratio</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_5$</td>
<td>&gt; 0.3550</td>
<td>0.9000</td>
<td>0.9000</td>
<td>9.00</td>
<td>0.9300</td>
</tr>
<tr>
<td>$Z_5$ (% predicted)</td>
<td>&gt; 131.5</td>
<td>0.6000</td>
<td>0.9000</td>
<td>6.00</td>
<td>0.8650</td>
</tr>
<tr>
<td>$R_5$</td>
<td>&gt; 0.3250</td>
<td>0.9000</td>
<td>0.8000</td>
<td>4.50</td>
<td>0.9350</td>
</tr>
<tr>
<td>$R_5$ (% predicted)</td>
<td>&gt; 122.4</td>
<td>0.6000</td>
<td>0.9000</td>
<td>6.00</td>
<td>0.8500</td>
</tr>
<tr>
<td>$R_{20}$</td>
<td>&gt; 0.2150</td>
<td>0.8000</td>
<td>0.9000</td>
<td>8.00</td>
<td>0.8800</td>
</tr>
<tr>
<td>$R_{20}$ (% predicted)</td>
<td>&gt; 95.55</td>
<td>0.8000</td>
<td>0.9000</td>
<td>8.00</td>
<td>0.8300</td>
</tr>
<tr>
<td>$X_5$</td>
<td>&lt; -0.1050</td>
<td>0.8000</td>
<td>0.9000</td>
<td>8.00</td>
<td>0.8950</td>
</tr>
<tr>
<td>$X_5$ (% predicted)</td>
<td>&gt; 1312</td>
<td>0.5000</td>
<td>0.9000</td>
<td>5.00</td>
<td>0.8600</td>
</tr>
<tr>
<td>$R_5-R_{20}$</td>
<td>&gt; 0.1100</td>
<td>0.6000</td>
<td>0.8000</td>
<td>3.00</td>
<td>0.8150</td>
</tr>
<tr>
<td>Fres</td>
<td>&gt; 22.44</td>
<td>0.8000</td>
<td>0.9000</td>
<td>8.00</td>
<td>0.8700</td>
</tr>
<tr>
<td>Ax</td>
<td>&gt; 0.9550</td>
<td>0.8000</td>
<td>0.9000</td>
<td>8.00</td>
<td>0.9300</td>
</tr>
<tr>
<td>Delta $X_5$</td>
<td>&gt; 0.0550</td>
<td>0.3000</td>
<td>0.9000</td>
<td>3.00</td>
<td>0.6100</td>
</tr>
<tr>
<td>SVC (%) predicted</td>
<td>&lt; 67.72</td>
<td>0.7000</td>
<td>0.9000</td>
<td>7.00</td>
<td>0.8200</td>
</tr>
<tr>
<td>FVC (%) predicted</td>
<td>&lt; 70.34</td>
<td>0.7000</td>
<td>0.9000</td>
<td>7.00</td>
<td>0.8100</td>
</tr>
<tr>
<td>FEV$_1$ (%) predicted</td>
<td>&lt; 60.22</td>
<td>0.7000</td>
<td>0.9000</td>
<td>7.00</td>
<td>0.8300</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>&lt; 67.17</td>
<td>0.6000</td>
<td>0.9000</td>
<td>6.00</td>
<td>0.7000</td>
</tr>
<tr>
<td>PEF (%) predicted</td>
<td>&lt; 28.67</td>
<td>0.4000</td>
<td>0.9000</td>
<td>4.00</td>
<td>0.5700</td>
</tr>
</tbody>
</table>

AUC: area under the curve; $Z_5$: total airway impedance; $R_5$: resistance at 5Hz; $R_{20}$: resistance at 20Hz; Fres: resonant frequency; Ax: area of reactance; $X_5$: reactance at 5Hz; $X_{20}$: reactance at 20Hz; $R_5-R_{20}$: peripheral airway resistance; SVC: slow vital capacity; FVC: forced vital capacity; FEV$_1$: forced expiratory volume in 1 second; PEF: peak expiratory flow.
Table 6. Levels of inflammatory markers in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=10)</th>
<th>PTBS (n=10)</th>
<th>PTBWS (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1 (ng/ml)</td>
<td>2.928[1.136-5.366]</td>
<td>3.134[1.847-3.600]</td>
<td>1.115[0.866-1.915]</td>
<td>0.0206*</td>
</tr>
<tr>
<td>TGF-β (ng/L)</td>
<td>173.7[120.2-264.5]</td>
<td>195.3[147.5-212.6]</td>
<td>141.1[111.8-150.5]</td>
<td>0.1006</td>
</tr>
</tbody>
</table>

Values expressed are median with inter-quartile range, analyzed by Kruskal-Wallis test.*p-value<0.05 statistically significant.

PTBS: post tuberculosis with sequelae; PTBWS: post tuberculosis without sequelae; MMP 1: matrix metalloproteinase-1; TGF β: transforming growth factor beta; INF γ: gamma interferon.
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-value<0.05, **p-value<0.01 and ***p-value<0.001 for intra group comparison.
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-value<0.05, **p-value<0.01, ***p-value<0.001 and ****p-value<0.0001 for intra group comparison.