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Multimodal Evaluation of Pulmonary Rehabilitation in Newly Diagnosed Tuberculosis Patients Using Respiratory Culture-Based Microbiome Profiling and Inflammatory Biomarkers

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ABSTRACT

OBJECTIVE Pulmonary rehabilitation (PR) plays a vital role in enhancing lung function and exercise capacity in individuals with pulmonary conditions. This study evaluated the clinical effectiveness of an 8-week PR program in newly diagnosed pulmonary tuberculosis (TB) patients through the assessment of lung function, exercise capacity, inflammatory biomarkers, and respiratory microbiome changes.

METHODS Sixty newly diagnosed TB patients were enrolled, with 30 completing the 8-week PR program. Pulmonary function (FEV_1, FVC, FEV_1/FVC) and exercise capacity (6-minute walk test, 6MWT) were measured before and after PR. Inflammatory biomarkers (CRP, IL-6, TNF-α) and sputum microbiome profiles were assessed. ROC curves and multiple regression models identified biomarkers that were predictive of functional improvement.

RESULTS PR led to significant improvements: FEV_1 increased from 2.50 ± 0.60 L to 3.10 ± 0.50 L ($p < 0.001$), FVC from 3.20 ± 0.70 L to 3.70 ± 0.60 L ($p = 0.002$), and 6MWT distance from 350.00 ± 40.00 m to 400.00 ± 50.00 m ($p = 0.003$). Oxygen saturation improved from 93.00 ± 2.00% to 95.00 ± 1.00% ($p = 0.022$). Inflammatory markers decreased significantly: CRP 18.00 ± 7.00 to 10.00 ± 5.00 mg/L ($p < 0.001$), IL-6 25.00 ± 8.00 to 15.00 ± 6.00 pg/mL ($p < 0.001$), and TNF-α 50.00 ± 20.00 to 32.00 ± 15.00 pg/mL ($p = 0.002$). ROC analysis identified CRP as the strongest predictor of improvement (AUC = 0.84). Multiple regression confirmed CRP as an independent predictor of lung function and exercise capacity gains.

CONCLUSIONS An 8-week PR program significantly improved lung function, exercise capacity, and reduced systemic inflammation in TB patients, with CRP emerging as a key biomarker for predicting rehabilitation outcomes.

KEYWORDS pulmonary rehabilitation, tuberculosis, inflammatory biomarkers, microbiome, lung function, exercise capacity

INTRODUCTION

Tuberculosis (TB) remains a significant global health concern, especially in low- and middle-income nations such as India. The World Health Organization (WHO) reports that India accounts for about 25% of the global TB burden, making it the leading contributor globally (1). Despite successful elimination of the pathogen with anti-tuberculosis therapy (ATT), many patients develop persistent pulmonary complications such as fibrosis, bronchiectasis, restrictive lung disease, and airflow obstruction (2). These post-TB sequelae significantly impact respiratory function and quality of life and remain inadequately addressed by current therapeutic approaches (3).

Pulmonary rehabilitation (PR) is a comprehensive intervention designed to enhance physical fitness, alleviate dyspnoea, and improve health-related quality of life in individuals with chronic respiratory conditions. PR programs typically combine supervised exercise training, patient education, nutritional advice, and psychological support. While substantial evidence supports the efficacy of PR in diseases such as chronic obstructive pulmonary disease (COPD) and post-COVID-19 lung dysfunction, its role in TB survivors remains underexplored. Emerging studies suggest that PR may help mitigate functional limitations and enhance recovery in patients recovering from pulmonary TB (4).

In resource-constrained settings, the implementation of practical, cost-effective assessment methods is essential. A comprehensive, non-invasive assessment approach integrating sputum culture analysis to monitor microbial colonization and respiratory microbiome alterations, along with inflammatory biomarkers including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) to evaluate systemic inflammation (5), offers a pragmatic framework to objectively monitor PR outcomes and guide long-term care in TB survivors.

Study objective

This study aimed to evaluate the clinical effectiveness of an 8-week PR program in newly diagnosed TB patients through assessment of lung function, exercise capacity, inflammatory biomarkers, and respiratory microbiome changes during standard anti-tuberculosis treatment.

METHODS

A prospective, interventional cohort study was conducted at PSG Hospital, Coimbatore, Tamil Nadu, India. Sixty individuals were recruited over the first 12 months of the 24-month study period. Newly diagnosed smear-positive or CBNAAT-positive pulmonary TB patients, aged between 18 and 60 years, with no history of previous TB treatment and capable of providing informed consent and adhering to study procedures were included. Patients with co-existing chronic lung diseases (COPD, ILD), active human immunodeficiency virus infection, multidrug-resistant or extensively drug-resistant TB, and those who were pregnant or lactating were excluded.

The study protocol was approved by the Institutional Ethics Committee (ECN: 1183/ICE/2023) and written informed consent was obtained from all participants.

All eligible patients were identified at the TB treatment clinic and invited to participate. Sixty patients were initially enrolled; however, only 30 patients completed the full 8-week PR program due to logistical constraints, transportation difficulties, and treatment adherence issues.

PR program

The PR program was initiated two weeks after commencing ATT to ensure patient stability. The structured 8-week program consisted of:

- Frequency: three supervised sessions per week
- Duration: 60 minutes per session
- Setting: hospital-based rehabilitation center
- Personnel: qualified physiotherapist and respiratory therapist
- Components:
  - Aerobic exercises: Walking, stationary cycling, and treadmill exercises (20-30 minutes), with intensity gradually increased based on individual tolerance using the Borg scale (6)
  - Breathing exercises: Diaphragmatic breathing, pursed-lip breathing, and inspiratory muscle training (15 minutes) to enhance lung expansion and respiratory control (7)
  - Strengthening exercises: Upper and lower limb resistance training using elastic bands and light weights (15 minutes) to improve overall physical endurance (8)

- Patient education: Weekly 30-minute sessions focusing on disease management, medication adherence, lifestyle modifications, and smoking cessation counselling

Assessment protocol

Assessments were conducted at two time points: baseline (pre-PR) and post-intervention (8 weeks).

Pulmonary Function Testing: Spirometry was performed using a calibrated digital spirometer (Model: Spirobank II, Mobile Industrial Robots (MIR), Rome, Italy) following American Thoracic Society guidelines (9). Measurements included forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and the FEV_1/FVC ratio. Three reproducible measurements were obtained, with the best values recorded.

Exercise capacity: the Six-Minute Walk Test (6MWT) was conducted following standardized protocols in a 30-meter corridor (10). Distance walked and oxygen saturation levels using pulse oximetry were recorded.

Quality of life: evaluation was performed using the St. George's Respiratory Questionnaire (SGRQ).

Inflammatory biomarkers: CRP, IL-6, and TNF–α were analyzed using validated enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) with established reference ranges. Blood samples were collected after 12-hour fasting and processed within 2 hours (11). These markers were selected based on their established roles in TB pathophysiology and systemic inflammation.

Sputum microbiological analysis: sputum samples were collected using standardized techniques and were processed within 4 hours. Aerobic and anaerobic bacterial cultures were performed using standard microbiological methods on blood agar and MacConkey agar plates, with identification confirmed by biochemical testing (12).

Statistical analysis

Data analysis was performed using SPSS version 25.0. Continuous variables are presented as mean ± standard deviation (SD). The paired t-test was used to compare pre- and post-PR outcomes for normally distributed continuous variables. The Chi-square test was utilized for categorical data comparisons. Pearson's correlation coefficient was calculated to examine relationships between inflammatory biomarkers and changes in pulmonary function parameters. Receiver operating characteristic (ROC) curves were constructed to evaluate the predictive value of inflammatory biomarkers for clinical improvement, defined as ≥10% increase in FEV_1 (13). The area under the curve (AUC) was calculated to assess diagnostic accuracy. Multiple linear regression analysis was performed to identify independent factors associated with improvements in lung function (FEV_1, FVC) and exercise capacity (6MWT). A p-value < 0.05 was considered statistically significant.

RESULTS

The study enrolled 60 patients with newly diagnosed pulmonary TB, of whom 30 completed the 8-week PR program. The demographic characteristics showed no significant differences between baseline and post-intervention assessments within the study cohort. The mean age of participants was 40.00 ± 12.00 years, with 18 males and 12 females. The body mass index (BMI) remained stable: 22.40 ± 2.10 kg/m² at baseline and 22.50 ± 2.00 kg/m² post-PR (p = 0.927). Smoking history was present in 10 patients, and showed no change during the study period (p = 1.000). TB severity distribution remained consistent, with 5 mild, 15 moderate, and 10 severe cases (Table 1).

The study demonstrated significant improvements in pulmonary function and exercise capacity following the 8-week PR intervention. FEV_1 increased from 2.50 ± 0.60 L pre-PR to 3.10 ± 0.50 L post-PR (p < 0.001), and FVC improved from 3.20 ± 0.70 L pre-PR to 3.70 ± 0.60 L post-PR (p = 0.002). Additionally, the FEV_1/FVC ratio improved from 78.00 ± 5.00% pre-PR to 84.00 ± 3.00% post-PR (p = 0.001). The 6MWT was used to evaluate exercise tolerance prior to and following the PR program. The distance walked in 6 minutes significantly increased from 350.00 ± 40.00 meters pre-PR to 400.00 ± 50.00 meters post-PR (p = 0.003). Oxygen saturation also improved, increasing from 93.00 ± 2.00% pre-PR to 95.00 ± 1.00% post-PR (p = 0.022) (Table 2).

Inflammatory biomarkers showed significant reductions following PR intervention. CRP levels diminished from 18.00 ± 7.00 mg/L before PR to 10.00 ± 5.00 mg/L after PR (p < 0.001), IL-6 levels reduced from 25.00 ± 8.00 pg/mL to 15.00 ± 6.00

Table 1. Demographics and baseline characteristics

Characteristic	Baseline (n = 30)	Post-PR (n = 30)	*p*-value
Age (years)	40.00±12.00	40.00±12.00	0.789
Gender (male/female)	18/12	18/12	1.000
BMI (kg/m²)	22.40±2.10	22.50±2.00	0.927
Smoking history (yes/no)	10/20	10/20	1.000
TB severity (mild/moderate/severe)	5/15/10	5/15/10	1.000

Values are expressed as mean ± SD
BMI, body mass index; PR, pulmonary rehabilitation; TB, tuberculosis

Table 2. Pulmonary function and exercise parameters

Parameter	Pre-PR (n = 30)	Post-PR (n = 30)	*p*-value
FEV_1 (L)	2.50±0.60	3.10±0.50	< 0.001*
FVC (L)	3.20±0.70	3.70±0.60	0.002*
FEV_1/FVC (%)	78.00±5.00	84.00±3.00	0.001*
6MWT distance (m)	350.00±40.00	400.00±50.00	0.003*
Oxygen saturation (%)	93.00±2.00	95.00±1.00	0.022*

Values are expressed as mean ± SD
FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; 6MWT, six-minute walk test; *$p < 0.05$ indicates statistical significance

Table 3. Inflammatory biomarkers and microbiome changes

Parameter	Pre-PR (n = 30)	Post-PR (n = 30)	*p*-value
Inflammatory biomarkers			
CRP (mg/L)	18.00±7.00	10.00±5.00	< 0.001*
IL-6 (pg/mL)	25.00±8.00	15.00±6.00	< 0.001*
TNF-α (pg/mL)	50.00±20.00	32.00±15.00	0.002*
Sputum microbial culture			
Streptococcus pneumoniae	10 (33.3)	3 (10.0)	0.034*
Pseudomonas aeruginosa	8 (26.7)	2 (6.7)	0.042*
Fusobacterium species	6 (20.0)	1 (3.3)	0.023*
Bacteroides fragilis	5 (16.7)	0 (0.0)	0.011*

Values are expressed as mean ± SD for continuous variables and n (%) for categorical variables.
*$p < 0.05$ indicates statistical significance
CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha

pg/mL ($p < 0.001$), and TNF-α levels declined from 50.00 ± 20.00 pg/mL to 32.00 ± 15.00 pg/mL ($p = 0.002$). Analysis of sputum microbiological profiles revealed favorable changes in respiratory microbiome composition. The presence of *Streptococcus pneumoniae* decreased from 33.3% pre-PR to 10% post-PR ($p = 0.034$), and *Pseudomonas aeruginosa* colonization reduced from 26.7% pre-PR to 6.7% post-PR ($p = 0.042$). Additionally, anaerobic pathogens such as *Fusobacterium* and *Bacteroides fragilis* also showed significant reductions, with *Fusobacterium* decreasing from 20% pre-PR to 3.3% post-PR ($p = 0.023$) and *Bacteroides fragilis* from 16.7% to 0% ($p = 0.011$) (Table 3).

Correlation analysis revealed strong negative associations between inflammatory biomarkers and functional improvements. Negative correlations were observed between the levels of CRP, IL-6, TNF-α, and improvements in FEV_1, FVC, and the 6MWT distance. Specifically, improvements in FEV_1 ($r = -0.63$), FVC ($r = -0.59$), and 6MWT distance ($r = -0.71$) were all statistically significantly negatively correlated with reductions in CRP (Table 4).

ROC analysis and multiple regression modelling identified inflammatory biomarkers as significant predictors of rehabilitation outcomes

Table 4. Correlation analysis between inflammatory biomarkers and functional parameters

Parameter	CRP	IL-6	TNF-α
FEV_1 (L)	-0.63*	-0.58*	-0.52*
FVC (L)	-0.59*	-0.54*	-0.49*
6MWT Distance (m)	-0.71*	-0.69*	-0.62*

Strong negative correlations were observed according to Cohen's guidelines (r > 0.50) (14), *$p < 0.05$ indicates statistical significance

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity, 6MWT, Six-Minute Walk Test

(Figure 1). CRP had the highest AUC of 0.84 (95%CI: 0.74–0.94), demonstrating a sensitivity of 82% and a specificity of 78% ($p < 0.001$). IL-6 and TNF-α had statistically significant predictive value, with AUCs of 0.81 (95%CI: 0.70–0.92) and 0.76 (95%CI: 0.64–0.88), respectively. In terms of FEV_1 enhancement, elevated baseline concentrations of CRP (β = -0.35, $p = 0.001$), IL-6 (β = -0.28, $p = 0.004$), and TNF-α (β = -0.22, $p = 0.009$) were substantially statistically correlated with less improvement. Likewise, for the 6MWT distance, heightened baseline levels of CRP (β = -0.41, $p < 0.001$), IL-6 (β = -0.33, $p = 0.002$), and TNF-α (β = -0.30, $p = 0.004$) were autonomous predictors of diminished improvements in exercise ability (Table 5).

DISCUSSION

This study demonstrates the beneficial effects of PR in enhancing pulmonary function, exercise capacity, and reducing systemic inflammation in newly diagnosed TB patients. These findings align with previous studies demonstrating significant improvements in lung function and overall health

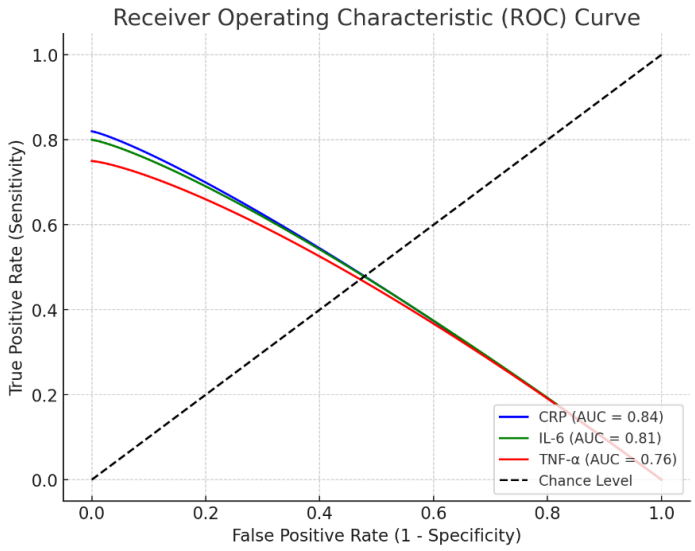

Figure 1. Receiver operating characteristic (ROC) curves for CRP, IL-6, and TNF-α in predicting clinical outcome

Table 5. ROC analysis and regression predictors

Analysis type	Biomarker	AUC (95% CI)	Sensitivity (%)	Specificity (%)	β Coefficient	*p*-value
ROC analysis	CRP	0.84 (0.74-0.94)	82.0	78.0	-	< 0.001*
	IL-6	0.81 (0.70-0.92)	80.0	75.0	-	< 0.001*
	TNF-α	0.76 (0.64-0.88)	75.0	72.0	-	0.002*
FEV_1 predictors	CRP	-	-	-	-0.35	0.001*
	IL-6	-	-	-	-0.28	0.004*
	TNF-α	-	-	-	-0.22	0.009*
6MWT predictors	CRP	-	-	-	-0.41	< 0.001*
	IL-6	-	-	-	-0.33	0.002*
	TNF-α	-	-	-	-0.30	0.004*

*$p < 0.05$ indicates statistical significance
ROC, receiver operating characteristic; FEV_1, forced expiratory volume in 1 second; 6MWT, Six-Minute Walk Test; AUC, area under curve; CI, confidence interval

following rehabilitation in patients with chronic respiratory diseases (15).

The mechanisms underlying improved lung function may involve several pathways. Enhanced respiratory muscle strength through targeted breathing exercises likely contributed to increased lung volumes and improved ventilation patterns. The observed improvements in FEV_1 and FVC suggest enhanced airway function and lung compliance, potentially resulting from reduced airway inflammation and improved respiratory mechanics (16). The structured aerobic training component may have enhanced cardiopulmonary efficiency, contributing to improved oxygen utilization and reduced ventilatory limitations during physical activity.

The significant reduction in inflammatory biomarkers (CRP, IL-6, TNF-α) following PR reflects the anti-inflammatory effects of appropriate regular exercise training. Physical exercise is known to reduce systemic inflammation through multiple mechanisms, including decreased production of pro-inflammatory cytokines and enhanced anti-inflammatory mediator release. In TB patients, this anti-inflammatory response may be particularly beneficial, as persistent inflammation contributes to long-term pulmonary complications including fibrosis and functional impairment (17).

The improvements in exercise capacity, as evidenced by enhanced 6MWT performance and oxygen saturation, likely resulted from combined cardiopulmonary adaptations. Enhanced oxygen delivery and utilization, improved respiratory muscle endurance, and reduced dyspnea perception contribute to better exercise tolerance. These physiological adaptations are particularly important for TB patients, who often experience significant functional limitations during recovery (18).

The observed beneficial changes in the respiratory microbiome, including reduced pathogenic bacterial colonization, suggest that PR may help restore microbial homeostasis. Regular physical activity has been associated with improved immune function and reduced susceptibility to respiratory infections (19). This finding is clinically relevant for TB patients, who remain at increased risk for secondary respiratory infections during treatment and recovery.

The strong correlations between inflammatory markers and functional improvements, along with the predictive value of these biomarkers, support their potential clinical utility. CRP emerged as the strongest predictor of rehabilitation success, which may facilitate personalized treatment approaches and outcome monitoring in TB patients undergoing PR (20).

Study limitations include the relatively small sample size and absence of a control group, which limits causal inference. The completion rate of 50% (30 of 60 enrolled) highlights implementation challenges in resource-limited settings. Future studies should include larger, randomized controlled trials with extended follow-up to assess long-term benefits and optimal PR protocols for TB patients.

CONCLUSION

An 8-week structured PR program significantly improved lung function, exercise capacity, and reduced systemic inflammation in newly diagnosed TB patients. CRP demonstrated the strongest predictive value for rehabilitation outcomes, suggesting its potential utility as a biomarker for monitoring treatment response. These findings support the integration of PR into standard TB care protocols to help optimize patient recovery and reduce long-term complications.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

L.V.: conceptualization, investigation, writing—original draft preparation; S.R.: conceptualization, methodology, supervision, project administration, funding acquisition, writing—review & editing; V.M.: methodology, validation, resources, investigation, writing—review & editing; D.M.: software, validation, formal analysis, data curation, writing—

review & editing; R.S.: formal analysis, data curation, visualization, writing—review & editing; M.K.I.: investigation, writing—original draft preparation, visualization.

All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy and ethical restrictions related to patient confidentiality.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (ECN: 1183/ICE/2023).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study. All participants received detailed information about the study procedures, potential risks, and benefits before providing written consent to participate in this research.

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