



# The Role of Matrix Metalloproteinase-9 in Lung Function Impairment in Patients with Post-Tuberculosis Sequelae

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## Abstract

**Background:** Post-tuberculosis (post-TB) sequelae are long-term pulmonary complications that persist despite completion of anti-tuberculosis treatment (ATT). These long-term effects frequently contribute to diminished pulmonary function and a lower quality of life. Matrix metalloproteinase-9 (MMP-9) is thought to be involved in the process of lung tissue injury among individuals recovering from tuberculosis. This study aimed to explore the correlation between serum MMP-9 levels and pulmonary function impairment in post-TB patients.

**Methods:** An observational case-control study was carried out at Dr. H. Abdul Moeloek Hospital in Lampung between April 2023 and March 2024. The study involved 35 post-TB patients (case group) and 35 healthy individuals (control group). Pulmonary function was evaluated using spirometry, while serum MMP-9 concentrations were measured by ELISA. The data were statistically analysed using the Mann-Whitney U test and Spearman's rank correlation.

**Results:** The post-TB group exhibited higher serum MMP-9 levels compared to the control group (1137.7±527.4 vs. 939.5±360.1), though the difference did not reach statistical significance. Post-TB patients showed reduced lung function, particularly in FEV<sub>1</sub>/FVC, FEV<sub>1</sub>, and FVC values. There was a significant correlation between MMP-9 levels and pulmonary function parameters, including pre-bronchodilator FEV<sub>1</sub>/FVC ( $r = -0.321$ ;  $P=0.007$ ), post-bronchodilator FEV<sub>1</sub>/FVC ( $r = -0.265$ ;  $P=0.027$ ), and %FEV<sub>1</sub> ( $r = -0.254$ ;  $P=0.034$ ).

**Conclusion:** Increased serum MMP-9 levels are associated with reduced lung function in post-tuberculosis patients, particularly in cases of obstructive impairment. MMP-9 has the potential to be used as a biomarker for lung injury in individuals with a history of tuberculosis.

**Keywords:** airway obstruction, lung function, MMP-9, post-tuberculosis, spirometry

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## INTRODUCTION

Post-tuberculosis (post-TB) sequelae represent chronic complications that arise following pulmonary tuberculosis, despite completion of appropriate anti-tuberculosis therapy (ATT). Many individuals with a history of TB continue to experience long-term respiratory symptoms that significantly impair their quality of life. These sequelae are characterized by structural and functional abnormalities involving the lung parenchyma, airways, pleura, vasculature, chest wall, and mediastinum. Epidemiological data indicate that adults with prior TB have a two- to four-fold increased

risk of developing chronic lung function impairment, including both obstructive and restrictive ventilatory defects.<sup>1</sup>

Although often underdiagnosed and overlooked over the past five decades, post-TB sequelae are estimated to affect 16–50% of cases worldwide.<sup>2</sup> A study by Daniels et al involving 324 post-TB patients reported that 48% exhibited impaired lung function, with 21% showing obstructive defects, 11.25% restrictive, and 2% mixed patterns.<sup>3</sup> The reduction in Forced Expiratory Volume in one second (FEV<sub>1</sub>) is thought to be associated with the formation of pulmonary cavities, leading to airflow obstruction.<sup>4</sup> In addition to obstruction, restrictive

abnormalities may also be present, which can be evaluated through forced vital capacity (FVC) parameters.<sup>5</sup>

The emergence of post-tuberculosis sequelae is strongly associated with the host's immune response to *Mycobacterium tuberculosis* (Mtb) infection.<sup>6-8</sup> Among the immunological mediators involved, Matrix Metalloproteinase-9 (MMP-9) is essential in the processes of tissue remodelling and inflammation.<sup>9</sup> Transcriptomic analyses of TB lesion biopsies have demonstrated significant upregulation of MMP-9 expression, contributing to progressive pulmonary tissue damage.<sup>10</sup>

Given this background, the present study aims to investigate the relationship between MMP-9 levels and pulmonary function impairment among patients with post-tuberculosis sequelae, using spirometric assessment to identify obstructive and restrictive lung function impairments.

## METHODS

This research was conducted as an observational study using a case-control design. The study was carried out at Dr. H. Abdul Moeloek Hospital from April 2023 to March 2024. The sample was divided into two groups: a case group and a control group. The case group comprised individuals with a history of pulmonary tuberculosis who had completed the full course of anti-tuberculosis therapy at community health centres (puskesmas) in the Bandar Lampung area. The control group included healthy individuals with no history of pulmonary tuberculosis and no respiratory symptoms, confirmed by a normal chest radiograph.

Sampling was carried out using a non-probability purposive sampling method. Inclusion criteria for the case group were a history of primary pulmonary TB, completion of anti-TB treatment for at least 6 months, treatment duration of 0–6 months, and age between 18 and 65 years. Inclusion criteria for the control group were healthy individuals aged 18–65 years, with no respiratory complaints, and normal chest radiological findings.

Individuals with active pulmonary TB, drug-resistant TB, extrapulmonary TB, human immunodeficiency virus (HIV) infection, autoimmune diseases, pregnancy, chronic obstructive pulmonary disease (COPD), asthma, sarcoidosis, stroke, diabetes mellitus, acute coronary syndrome (ACS), and pulmonary malignancies were excluded from the sample.

Participants were instructed to perform both slow vital capacity (VC) and forced vital capacity (FVC) maneuvers using a spirometer (Chestgraph HI-105). The evaluated parameters included the FEV<sub>1</sub>/FVC ratio, FEV<sub>1</sub>, FVC, and forced expiratory flow between 25% and 75% of lung volume (FEF<sub>25-75%</sub>). Spirometry was conducted following the standards set by the American Thoracic Society (ATS) and the European Respiratory Society (ERS).

A 3 mL sample of peripheral venous blood was drawn under aseptic conditions to assess MMP-9 levels. Plasma was then separated and stored at –20°C. The concentration of serum MMP-9 was determined using the Human MMP-9 Quantikine ELISA Kit (R&D Systems, Catalog No. DMP900), which has a detection range of 13.2 to 105 ng/mL.<sup>11</sup>

The quantification of MMP-9 was carried out following the manufacturer's instructions. Briefly, all reagents, standards, and samples were prepared as directed. Each well received 100 µL of either standard or sample and was incubated at room temperature for 2.5 hours. Next, 100 µL of the biotinylated antibody solution was added and incubated for 1 hour. This was followed by the addition of 100 µL of the prepared Streptavidin solution and a 45-minute incubation. Subsequently, 100 µL of the TMB One-Step Substrate Reagent was added, with a 30-minute incubation. Finally, 50 µL of Stop Solution was added, and absorbance was immediately measured at 450 nm.<sup>11</sup>

The data were analysed using the Mann–Whitney U test to compare MMP-9 levels and pulmonary function between the case and control groups. Additionally, the relationship between MMP-9 levels and lung function parameters in post-tuberculosis patients was evaluated using Spearman's rank correlation (Spearman's rho). A

value of *P* less than 0.05 was considered statistically significant.

The study received ethical approval from the Health Research Ethics Committee of Dr. H. Abdul Moeloek Hospital, with the approval number 3637/UN26.18/PP.05.02.00/2022.

**RESULTS**

Initially, 86 samples were obtained; however, several were excluded due to damage or failure to fulfill the inclusion and exclusion criteria. Consequently, 70 samples were eligible for the final analysis, consisting of 35 participants in the case group and 35 in the control group.

The majority of participants in the case group were in the early elderly age category (46–55 years), while most participants in the control group were in late adolescence (17–25 years). Both groups were predominantly male. Comorbidities found in post-tuberculosis patients included hypertension, diabetes mellitus, asthma, cardiovascular disease, and HIV. Both groups had a history of light smoking (low Brinkman Index), normal oxygen saturation levels, and a Modified Medical Research Council (mMRC) dyspnea score of zero, indicating no breathlessness during daily activities. Nutritional status was mostly normal in both groups; however, 31% of post-TB respondents were undernourished. Additionally, 48.6% of post-TB respondents had a poor performance on the Six-Minute Walk Test (6MWT), indicating reduced functional capacity.

The MMP-9 is a key mediator of extracellular matrix degradation and pulmonary remodelling. This study compares MMP-9 levels in post-tuberculosis sequelae patients and healthy individuals to evaluate the role of residual inflammation in persistent lung damage after tuberculosis, as well as the potential of MMP-9 as a prognostic biomarker. The analysis of MMP-9 serum levels showed that post-TB patients had higher MMP-9 concentrations compared to the control group (1137.7±527.4 ng/mL vs. 939.5±360.1 ng/mL).

Spirometry examination demonstrated that the majority of participants in the healthy group had

normal lung function. In contrast, among post-TB patients, 42.9% patients exhibited reduced FEV<sub>1</sub>/FVC values in the pre-BD phase, and 31.4% patients in the post-bronchodilator (post-BD) phase. Additionally, 22.9% patients showed a decrease in FEV<sub>1</sub>, 48.6% patients had reduced FVC values, and 48.6% patients exhibited decreased FEF<sub>25%</sub>, FEF<sub>50%</sub>, and FEF<sub>75%</sub> values. The data also revealed a pattern suggesting that higher MMP-9 levels were associated with reduced lung function. This suggests a potential inverse relationship, where higher MMP-9 expression corresponds to greater impairment in lung function.

Table 1. Lung Function in the case and control groups

Lung Function	Groups		P
	Control	Case	
FEV <sub>1</sub> /FVC pre-BD (mean±SD)	89.9±6.1	77.9±14.3	
Normal (>75%)	35 (100.0%)	20 (57.1%)	0.0001
Obstruction (<75%)	0 (0.0%)	15 (42.9%)	
FEV <sub>1</sub> /FVC post-BD (mean±SD)	88.7±7.5	80.8±11.9	
Normal (>75%)	34 (97.1%)	24 (68.6%)	0.004
Obstruction (<75%)	1 (2.9%)	11 (31.4%)	
FEV <sub>1</sub> (mean±SD)	77.1±14.2	47.1±21.3	
Normal (>75%)	23 (65.7%)	4 (11.4%)	0.004
Mild (60–74%)	7 (20%)	4 (11.4%)	
Moderate (30–59%)	5 (14.3%)	19 (54.3%)	
Severe (<30%)	0 (0.0%)	8 (22.9%)	
FVC (mean±SD)	73.1±14.3	50.2±21.9	
Normal (>80%)	18 (51.4%)	1 (2.9%)	0.0001
Mild (60-79%)	9 (25.7%)	3 (8.6%)	
Moderate (30-59%)	5 (14.3%)	14 (40%)	
Severe (<30%)	3 (8.6%)	17 (48.6%)	
FEF <sub>25%</sub> , 50%, and 75% (mean±SD)	90.4±31.7	50.6±33.7	
Normal	35 (100.0%)	18 (51.4%)	0.0001
Decreased	0 (0.0%)	17 (48.6%)	

Spearman correlation analysis revealed a significant relationship between MMP-9 levels and lung function parameters. Notably, a moderate negative correlation was found between MMP-9 and the FEV<sub>1</sub>/FVC ratio in the pre-BD phase (*r* = −0.321; *P*=0.007), the post-BD phase (*r* = −0.265; *P*=0.027), and %FEV<sub>1</sub> predicted (*r* = −0.254; *P*=0.034). These results indicate that higher MMP-9 levels may be associated with impaired lung function, particularly suggestive of obstructive airway conditions, as reflected by reduced FEV<sub>1</sub>/FVC and FEV<sub>1</sub> values.

Table 2. Analysis of Differences in Lung Function Between Healthy Controls and Post-Tuberculosis Patients.

Lung function	MMP-9 (ng/mL)		<i>r</i>	<i>P</i>
	Control	Case		
FEV <sub>1</sub> /FVC pre-BD				
Normal (>75%)	939.5±360.06	1171.0±517.8	-0.321	0.007
Obstruction (<75%)	0	1130±556.5		
FEV <sub>1</sub> /FVC post-BD				
Normal (>75%)	954.9±359.17	1064.7±520.27	-0.265	0.027
Obstruction (<75%)	685.4±377.88	1316.9±542.17		
FEV <sub>1</sub>				
Normal (>75%)	901.6±321.4	1071.0 ± 597.9	-0.254	0.034
Mild (60–74%)	975.8±379.5	947.1 ± 551.4		
Moderate (30–59%)	1063.4±536.8	1122.8 ± 521.1		
Severe (<30%)	0	1279.8 ± 575.9		
FVC				
Normal (>80%)	865.1±320.4	752.9 ± 242.0	-0.200	0.097
Mild (60-79%)	965.4±353.5	1248.0 ± 597.4		
Moderate (30-59%)	993.6±463.5	1078.6 ± 482.0		
Severe (<30%)	0	1249.6 ± 615.2		
FEF25%				
Normal (>75%)	951.4±289.6	1078.7 ± 749.6	-0.077	0.527
Obstruction (<75%)	919.5±468.9	1143.3 ± 518.1		
FEF 50%				
Normal (>75%)	933.2±329.5	923.2 ± 462.9	-0.214	0.075
Obstruction (<75%)	947.9±409.1	1201.3 ± 536.3		
FEF 75%				
Normal (>75%)	928.8±311.0	1132.3 ± 590.4	-0.235	0.050
Obstruction (<75%)	960.1±454.5	1138.4 ± 529.5		

## DISCUSSION

The majority of post-TB patients in our study sample were male and within the middle-aged to early elderly age group. These results align with data from the World Health Organization (WHO), which indicate that the highest global incidence of tuberculosis occurs among individuals aged 45 to 55 years, with a peak occurring in those over 65 years of age—particularly in the Western Pacific, Eastern Mediterranean, and Southeast Asia regions.<sup>12</sup> This finding is also consistent with a study conducted by Irawati in 2013 at Soedarso Hospital, Pontianak, which reported that the majority of post-tuberculosis cases occurred in older adults aged 55–64 years and were predominantly male.<sup>13</sup>

This study demonstrated that post-TB patients had poor nutritional status and reduced respiratory quality, as reflected by higher mMRC scores and lower Six-Minute Walk Test (6MWT) performance. These findings are in agreement with the study conducted by Wagnew et al, which reported that TB

patients frequently experience appetite loss, nutrient and macronutrient malabsorption, all of which can impair the healing process and increase the risk of mortality.<sup>14</sup>

The study revealed a significant disparity in lung function between healthy individuals and those with a history of tuberculosis (post-TB). These results are in line with the findings of Manji et al, who reported that post-TB patients commonly exhibit lung function abnormalities, including obstructive, restrictive, and mixed ventilatory defects.<sup>15</sup>

A study conducted by Shanmugasundaram et al also found that individuals who had previously suffered from tuberculosis (post-TB patients) showed significantly lower levels of FVC, FEV<sub>1</sub>, and the FEV<sub>1</sub>/FVC ratio compared to healthy individuals. This suggests that tuberculosis can have lasting effects on lung function, even after recovery.<sup>16</sup>

Common residual manifestations observed in post-TB individuals include obstructive abnormalities, indicated by a reduced FEV<sub>1</sub>/FVC ratio, and restrictive defects, reflected in decreased FVC

values. The host immune response to Mtb infection may lead to pulmonary remodelling, which can result in persistent airway obstruction and restrictive respiratory patterns.<sup>16</sup>

This study demonstrated the presence of obstructive impairment in post-TB patients, as evidenced by a decrease in mean predicted %FEV<sub>1</sub>/FVC and predicted %FEV<sub>1</sub>. These findings are in line with the results reported by Rhee et al in South Korea, which showed that a reduction in mean FEV<sub>1</sub> following tuberculosis treatment was comparable to the decline observed in patients with COPD.<sup>17</sup> Similarly, research by Ralph et al in Indonesia reported that moderate to severe airway obstruction was already present at the initiation of TB treatment.<sup>18</sup>

Furthermore, research by Pradipta et al at Hasan Sadikin General Hospital found that 83.4% of TB patients who received category 1 anti-tuberculosis treatment experienced a decline in FEV<sub>1</sub>, and 76.7% showed a reduction in FVC.<sup>19</sup> This study also demonstrated the presence of restrictive impairment in post-TB patients, as indicated by a reduction in mean predicted FVC%. These findings align with those of Rhee et al, who reported that restrictive respiratory defects can persist even after tuberculosis treatment has been completed.<sup>17</sup>

This study also found that the mean serum MMP-9 level in post-TB patients was higher than in healthy controls, but the difference was not statistically significant. This finding is consistent with the results reported by Warriar et al, who also observed elevated serum MMP-9 levels in individuals after completing TB treatment. However, research specifically examining MMP-9 levels in post-TB patients is still limited. Most existing studies have concentrated on active TB cases, where elevated MMP-9 levels are more commonly documented.<sup>20</sup>

For instance, Sheen et al found that tuberculous pleural effusions contained significantly higher concentrations of MMP-9 compared to malignant or systemic pleural effusions.<sup>21</sup> These elevated levels were associated with granuloma formation, suggesting a distinct role for MMP-9 in the

inflammatory response to TB infection.<sup>22</sup> In healthy individuals, MMP-9 levels may transiently increase due to physiological immune responses, intense physical activity, environmental exposure, aging-related low-grade inflammation, circadian variation, and pre-analytical factors. These increases are typically mild, reversible, and non-progressive, distinguishing them from the sustained elevation observed in pathological conditions such as post-tuberculosis lung sequelae.<sup>22-24</sup>

Matrix metalloproteinases (MMPs) are key mediators in the inflammatory response and tissue destruction in tuberculosis. MMP-9, in particular, has the ability to break down components of the extracellular matrix, a process that plays a key role in the lung tissue damage caused by tuberculosis. By contributing to the degradation of structural proteins, MMP-9 facilitates tissue remodelling and cavity formation, which are hallmark features of TB-related pulmonary injury.<sup>4</sup> Mtb infection in macrophages and monocytes stimulates the secretion of MMP-9. Additionally, Mtb-infected mononuclear cells release TNF- $\alpha$  and IL-1 $\beta$ , which further promote MMP-9 expression.<sup>22</sup>

This study demonstrated a significant correlation between serum MMP-9 levels and key lung function parameters—specifically post-bronchodilator FEV<sub>1</sub>/FVC and FEV<sub>1</sub>—with a moderate strength of association. This indicates a potential role for MMP-9 in the development of obstructive airway disease among post-TB patients, suggesting that elevated MMP-9 may contribute to long-term pulmonary impairment following TB infection. A review by Ravimohan et al supported this mechanism, stating that MMP-9 contributes to cavity formation in TB, which may lead to abnormal tissue remodelling, resulting in atelectasis and respiratory impairment, particularly in the form of airway obstruction.<sup>4</sup>

## LIMITATIONS

This study faced certain limitations, particularly regarding the homogeneity of age and sex within the sample population. This lack of

diversity may have influenced the results and contributed to the absence of statistically significant differences in serum MMP-9 levels between the post-TB and control groups. Greater variability in demographic characteristics might have provided a clearer understanding of the relationship between MMP-9 and lung function outcomes.

## CONCLUSION

In conclusion, this study showed that post-TB patients had higher serum MMP-9 levels than healthy individuals, and that elevated MMP-9 was significantly associated with reduced lung function in this group. These findings highlight the potential role of MMP-9 in the long-term respiratory complications following tuberculosis infection.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

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