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ORIGINAL STUDY

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Association Between TLR2 Gene Mutations and Pulmonary Function in Patients with Pulmonary Tuberculosis

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Abstract

Background: Toll-like receptor 2 (TLR2), which is expressed on myeloid cells, detects harmful substances from invading infections or host-damaged tissues, leading to inflammation.

Objectives: Evaluation of the effects of TLR2 gene mutation on pulmonary function in pulmonary tuberculosis.

Methods: A prospective observational study lasting from January 2024 to December 2024 carried out on a sample of Iraqi patients from Kirkuk city who attended TB units at primary health care districts. Spirometry was done for all patients 2 months after the onset of anti-TB treatment and again after 6 months at the end of the treatment course. Blood samples from all patients were collected for TLR2 genetic analysis.

Results: The results showed a significant difference in FEV1 (44 ± 14.6), (59.5 ± 25.6) between mutants and non-mutants respectively, after 2 months of starting anti-TB treatment, while there was a significant difference in FEV1/FVC (98.2 ± 8.1), (107.7 ± 11.1) between mutants and non-mutants respectively, after 6 months of starting anti-TB treatment.

Conclusion: TLR2 mutations in PTB provide new insights into future research and treatment plans as a possible biomarker for respiratory dysfunction.

Keywords: TLR2, Pulmonary function, Pulmonary tuberculosis

1. Introduction

Even while doctors still view tuberculosis (TB) as a classical infectious disease, our understanding of the disease's clinical manifestations has changed over the last two millennia (Ali et al., 2020). Nearly half of the 8 to 10 million newly diagnosed cases of lung disease that arise from this pool each year, according to estimates from the World Health Organization (WHO), are communicable varieties [1]. In TB-endemic countries like Iraq, the rise of multiple drug resistance (MDR) and extensive drug resistance (XDR) has made tuberculosis a serious issue. 3One of those aspects that has been the subject of much recent research is the relationship between genetic polymorphisms in Toll-like receptor (TLR) family genes and the likelihood of acquiring pulmonary tuberculosis. Through the detection of harmful chemical patterns like as surface lipoproteins, phosphoteichoic acid, and lipopolysaccharide, the innate immune response to a range of pathogenic pathogens depends heavily on TLRs [2].

Type 1 transmembrane proteins known as toll-like receptors (TLRs) are members of the broader family of pattern recognition receptors. They are able to identify a wide range of molecular signals, including those linked to infections and endogenous damage, as well as patterns linked to damage and pathogens. The only TLR that can combine with more than two other TLR types to generate functional heterodimers is TLR2. Furthermore, a variety of non-TLR molecules interact with TLR2 [3].

Toll-like receptor 2 (TLR2), which is expressed on myeloid cells, detects harmful substances from invading infections or host-damaged tissues, leading

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to inflammation [4]. Genetic differences in toll-like receptor 2 (TLR2) affect the immune response to ESAT-6, an early secreted antigenic protein produced by Mycobacterium TB, in patients with pulmonary tuberculosis. The recognition of Mtb is associated with the expression of TLRs, pattern recognition receptors that are essential for protecting the host from pathogenic diseases, and the activation of inflammasomes, which are innate immune system receptors and sensors that regulate the activation of caspase-1 (interlukine-1 converting enzyme) and induce inflammation in response to infectious microorganisms and molecules derived from host proteins. Relationships between TB risk and gene polymorphisms in additional TLR (TLR1, 4, 6, 8, 9, and 10) genes have also been studied [5].

Spirometry, another name for the pulmonary function test, provides a numerical evaluation of the physiological characteristics of the respiratory system, which includes the lungs and chest wall [6]. Office-based spirometry can provide diagnostic data that is just as reliable and practical as that obtained from pulmonary function lab testing. Spirometry can be used to track the course of lung disease and the effectiveness of treatment [7] significant number of TB survivors' spirometry studies revealed a high prevalence of lung function impairment. The majority of patients had either restrictive pulmonary disease (low FVC), obstructive lung disease (low FEV1/FVC ratio), or mixed obstructive/restrictive pulmonary illness [8]. Reduced airflow and a loss in total lung capacity are the outcomes of the caseation, cavitation, and fibrosis mechanisms in tuberculosis. Daily tasks could be hindered by the substantial limitations TB patients may have in their ability to tolerate physical activity [9].

The aim of this study is to evaluate the effects of TLR2 gene mutation on pulmonary function in pulmonary tuberculosis

2. Materials and methods

2.1. Patients and study deasign

This is a cross-sectional study carried out between January 2024 and December 2024 at Tuberculosis Units in the Primary Healthcare Districts in Kirkuk Province. 65 patients with pulmonary tuberculosis (40 males and 25 females) diagnosed on the basis of history, clinical examination, chest radiography, and either GeneXpert or a direct smear sputum examination.

Weight in kilograms and length in meters squared were used to compute body mass index (BMI; Kg/m2). Spirometry was performed 2 months after starting anti-TB treatment and repeated after 6 months at the end of the treatment course using MIR Minispir PC-Based Spirometer with a disposable turbine and mouthpiece for each patient.

The study excluded participants with asthma, chronic obstructive pulmonary disease, interstitial lung disease, congestive heart failure, stroke, neuromuscular disease, smokers and ex-smokers, patients who were contraindicated for spirometry, and patients taking long-term medications that may cause pulmonary toxicity- such as amiodarone, bleomycin, cyclophosphamide, methotrexate, sulfa, and nitrofurantoin.

Reduced FEV1, normal (or decreased) VC, normal or decreased FVC, and a lowered FEV1/FVC ratio were considered obstructions, whereas reduced FVC and a normal-to-high FEV1/FVC ratio were considered restrictions. The degree of obstruction was categorized as mild ($\geq$80%), moderate (50–79%), severe (30–49%), or very severe (<30%). Both forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) are decreased in restrictive lung disease; however, the FVC reduction is greater than the FEV1 decline, leading to an FEV1/FVC ratio greater than 80% [10]. Seven millilitres of venous blood were drawn once from each patient. Two sets of tubes contained labels.

For the TLR2 (rs5743708) gene mutation study, tubes contained ethylenediamine tetra-acetic acid (EDTA) and two millilitres of blood. Geneaid-Taiwan used an extraction kit called Presto Mini gDNA to extract DNA from blood in accordance with the manufacturer's instructions. Following DNA extraction, Nano-Drop was used to assess the concentration of DNA (ng/μl) and confirm the DNA extracted from the DNA samples. The absorbance at (260/280 nm) was used to measure DNA purity. A dried powder of the primer (Table 1) was used (Macro Gene; South Korea).

Functional and stock solutions were created according to the manufacturer's instructions. The DNA template, primers, master mix, and free nuclease water were combined to a total volume of 20 μl volume to create the PCR reaction mixture. The components of the PCR master mix were centrifuged in a microcentrifuge for three minutes at 3000 rpm. A PCR thermocycler (Applied Bio system, Singapore) was then used to process each sample. Agarose gel electrophoresis was employed to analyze the PCR product using an ultraviolet (UV) transilluminator.

2.2. Statistical analysis

Microsoft Office Excel 2016 with IBM's Statistical Package for Social Sciences (SPSS) version 27 was used for analysis. Mean $\pm$ standard deviation is used to display the numerical data. For comparisons between two independent groups (Mutant

Table 1. TLR2 gene primer design.

Gene	Primer sequence (5′-3′)
TLR2 rs5743708	Inner Forward 08 AGCGCTTCTGCAAGCTTCG Inner Reverse 08 AGGTAGGTCTTGGTGTTCATTATCTGCT Outer Forward 08 CCTACTGGGTGGAGAACCTTATGGT Outer Reverse 08 AGTCCTCAAATGACGGTACATCCAC Annealing 63 °C Product size for G allele: 235 Product size for A allele: 331 Product size of two outer primers: 519

vs. Non-mutant), an independent samples t-test was used. A p-value of < 0.05 was considered statistically significant. While our primary analysis focused on group comparisons at individual timepoints, we acknowledge that multiple comparisons were made. Therefore, we also performed a bivariate correlation analysis (Point-Biserial Correlation for the dichotomous genetic variable) to assess the relationship between the TLR2 mutation status and the change in spirometric parameters from month 2 to month 6.

2.3. Ethical Approval

The study was approved by the IRB committee at the College of Medicine, Baghdad University (1638 on 20/12/2023), and patients provided written informed consent to participate.

3. Results

The age range of the patients was 22–69 years. The mean patient age was 38 ± 12 years. There were 65 patients (40 males and 24 females) with a male-to-female ratio of 1.6:1. Regarding the occupation distribution of the studied sample, there were (10, 15.4%) housewives, (22, 33.8%) freelancers, (18, 27.8 %) employees, (5, 7.6%) students and (10, 15.4%) retired patients. The mean BMI of the patients was 21 ± 1.9. Fig. 1 shows the distribution of patients among BMI categories; most of the patients had a normal BMI (47, 59.5%).

Fig. 2 shows the chronic disease distribution among study patients, (15, 23%) had different comorbidities, most of whom were diabetic (10, 15.4%). Among the studied samples, there were (10, 15.4%) patients who had TLR2 mutations (Fig. 3).

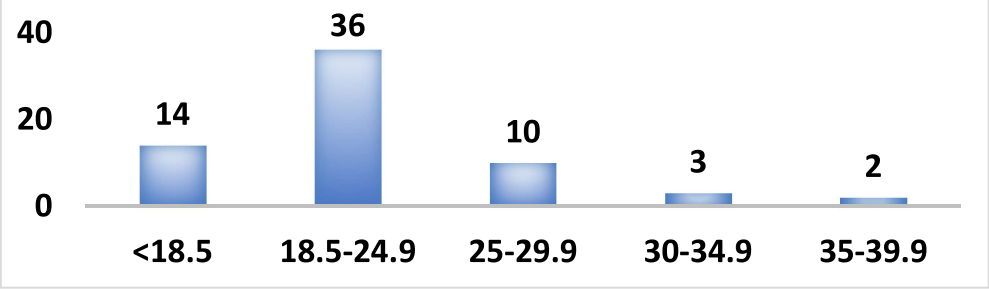

Fig. 1. Patients' distribution among BMI categories.

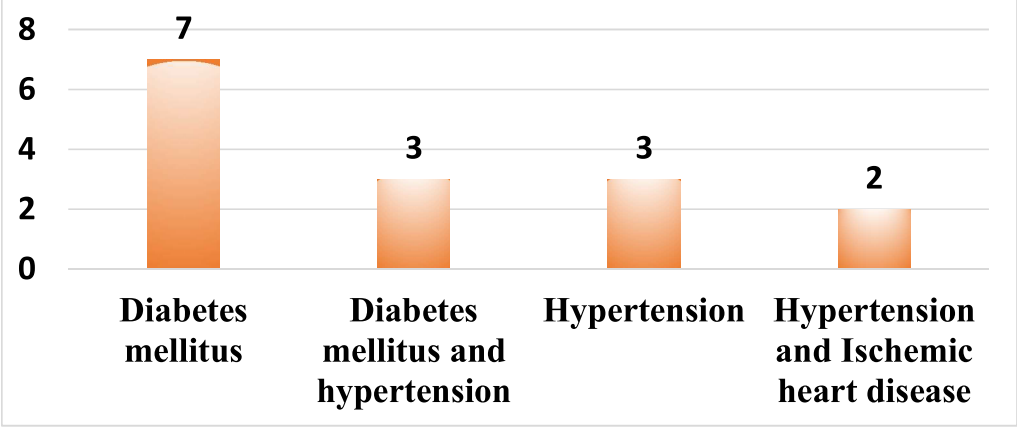

Fig. 2. Comorbidities distribution.

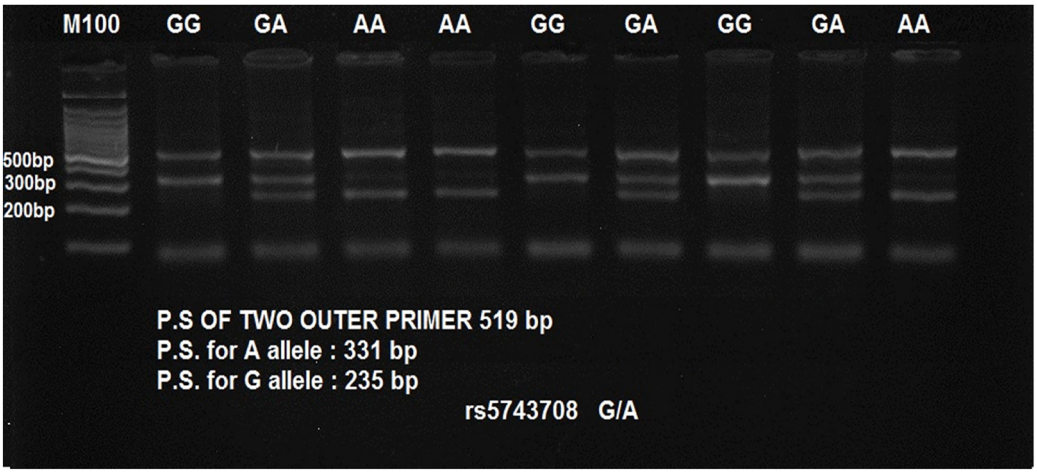

Fig. 3. TLR2 (rs5743708) PCR product (519 bp) on Agarose gel electrophoresis.

Table 2. FVC, FEV1 and FEV1/FVC% in the TLR2 Gene mutant and non-mutant patients with patterns of spirometry results after 2 months of starting anti-TB and after 6 months of starting anti-TB treatment.

After 2 months from starting anti TB treatment				
	FVC Mean ± SD	FEV1 Mean ± SD	FEV1/FVC % Mean ± SD	Pattern
Mutant (n: 10)	51.5 ± 18.4	44 ± 14.6*	107.2 ± 8.4	Restrictive
Not Mutant (n: 55)	57.6 ± 33.6	59.5 ± 25.6*	103.2 ± 14.3	Restrictive
P value	P > 0.05	P < 0.05	P > 0.05	
After 6 months from starting anti TB treatment				
	FVC Mean ± SD	FEV1 Mean ± SD	FEV1/FVC % Mean ± SD	Pattern
Mutant (n: 10)	72.2 ± 13.2	95.1 ± 8.3	98.2 ± 8.1*	Mixed
Not Mutant (n: 55)	67.5 ± 9.2	94.1 ± 9.3	107.7 ± 11.1*	Restrictive
P value	P > 0.05	P > 0.05	P < 0.05	

FVC: Forced Vital Capacity.
FEV1: Forced Expiratory Volume in 1st.Second.
*Significant differences at level P < 0.05.

Table 2 shows FVC, FEV1 and FEV1/FVC means in mutant and non-mutant patients with PTB and the pattern of results.

4. Discussion

In patients receiving anti-tuberculosis treatment, the study provides a thorough examination of demographic traits, BMI distribution, comorbidities, and the effect of TLR2 mutations on pulmonary function. The results provide insights into the clinical and genetic factors impacting TB-related pulmonary recovery by highlighting notable disparities in spirometry outcomes between patients with and without a TLR2 mutation.

The sex distribution of PTB patients in this study is in line with new research showing that males are more likely than females to suffer from some form of chronic illness, perhaps as a result of hormonal and lifestyle variations [11]. Metabolic health and pulmonary function were significantly predicted by BMI. The prevalence of overweight and obesity may worsen chronic respiratory illnesses by reducing lung compliance and causing systemic inflammation.

The high rates of diabetes and hypertension in this population reflect global trends of higher rates of non-communicable diseases, particularly among middle-aged individuals. Diabetes has been demonstrated to impairs lung function by compromising the immune response and causing microvascular damage [12].

Although the exact causes and severity range of pulmonary tuberculosis in patients remain unknown, one notion is that each person has a different mechanism of Toll-like receptors, including TLR2.

Examining the processes of innate immunity brought on by pathogen invasion in the host via TLRs is essential. A signaling cascade that recruits several proteins, including MyD88 as a common adapter, triggers TLR stimulation. This cascade also recruits interleukin-1 receptor-associated kinases and tumor necrosis factor receptor associated factor-6, which activates mitogen-activated protein kinases and causes nuclear translocation of nuclear factor-kB and activated activator protein-1. The activation of nuclear factor-kB by the signaling cascade results in the release of pro-inflammatory cytokines, some of which will dictate the illness manifestation, which is mostly brought on by the way tissue is destroyed. Single nucleotide polymorphisms in TLRs are thought to help identify M. tuberculosis. Variations in the TLR gene may account for distinct disease presentations caused by distinct innate immune responses to M. tuberculosis. The many kinds of tuberculosis severity reflect a balance between germs and host immune responses [13].

While our study focuses on the role of TLR2, it is critical to acknowledge that post-TB lung dysfunction is a multifactorial process. Beyond genetic predisposition in pattern recognition receptors, the extent of lung damage is heavily influenced by the severity of the initial cavitary disease, the host's inflammatory response leading to fibrosis, and potential secondary bacterial infections [9]. Furthermore, the development of bronchiectasis and bronchial stenosis due to granulomatous involvement of the airways is a well-documented causes of persistent obstructive defects [14]. Our findings on TLR2 should therefore be interpreted as identifying one important genetic component within this complex interplay of host and pathogen factors, rather than the sole determinant.

The potential biological mechanism linking TLR2 mutations to altered lung repair may lie in the dysregulation of the immune response. TLR2 recognizes Mycobacterium tuberculosis lipoproteins, initiating a signaling cascade that leads to the production of pro-inflammatory cytokines and chemokines (e.g., TNF-α, IL-6, IL-12) crucial for forming protective granulomas. A loss-of-function mutation, such as rs5743708, could impair this signaling, potentially leading to an inadequate initial immune response that allows for broader tissue damage. Conversely, this same impairment might later result in a diminished profibrotic response during the healing phase, potentially explaining the more significant improvement in FVC observed in mutants. This biphasic role—promoting initial control but also driving fibrosis—aligns with the concept of TLR2 acting as a 'double-edged sword' in TB pathogenesis, as discussed in other populations [13, 15].

At two months, the restrictive pattern predominated in both groups, which is consistent with lung damage linked to tuberculosis (TB) [14]. By six months, non-mutants continued to exhibit restriction (FEV1/FVC: 107.7 ± 11.1,), but mutants changed to a mixed pattern (FEV1/FVC: 98.2 ± 8.1). TLR2 mutations may affect parenchymal healing, potentially by increased fibroblast activity, as seen in the mutants' improved FVC (+20.7 points vs. +9.9) as shown in (Fig. 4). On the other hand, non-mutants with consistently high FEV1/FVC indicate continuous airway blockage, which calls for long-term observation.

15.4% of patients had the TLR2 (rs5743708) mutation, which is significant considering that TLR2 plays a part in innate immunological responses to Mycobacterium tuberculosis (Ruifeng et al., 2023). At two months, the mutant group's FEV1 was considerably lower (44 ± 14.6 vs. 59.5 ± 25.6,), indicating compromised early pulmonary function. By six months, however, mutants showed a remarkable FEV1 recovery (+51.1 points compared to +34.6 in non-mutants) as shown in Fig. 6, suggesting a strong but delayed compensatory mechanism. This is consistent with research demonstrating that TLR2 polymorphisms may change the dynamics of cytokines, causing

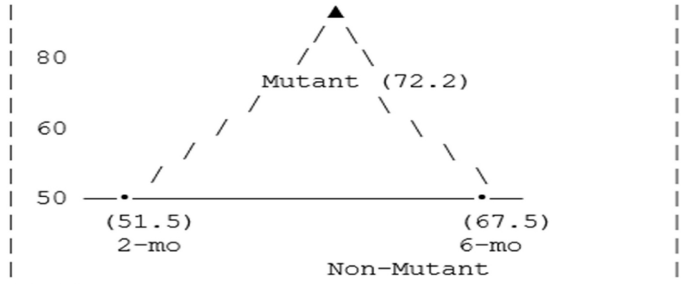

Fig. 4. FVC Trends (2 & 6 months).

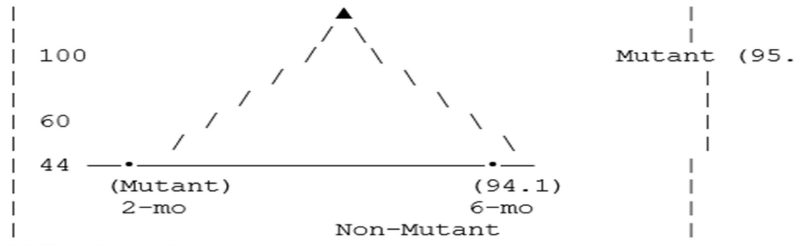

Fig. 5. FEV1 trends (2 & 6 months).

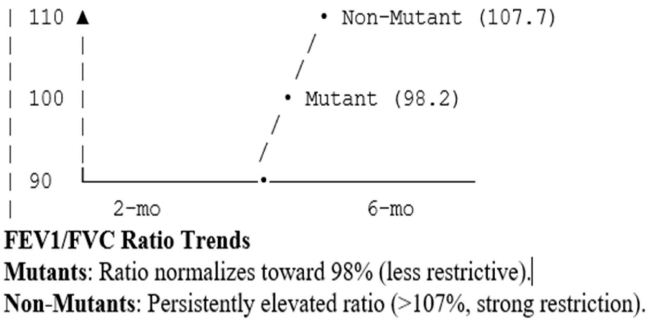

Fig. 6. FEV1/FVC ratio trends (2 & 6 months).

inflammation to worsen at first but thereafter encouraging repair [17].

Over a six-month period, the graphs (Figs. 4 to 6) show notable variations in the recovery of lung function between the mutant and non-mutant groups. Trends in FVC: Mutants displayed a significant improvement (+20.7 points), indicating a quick recovery of lung volume. Slower development (+9.9 points) was made by non-mutants, suggesting less effective forced vital capacity repair. Trends in FEV1: Mutants showed significant airflow restoration with a dramatic FEV1 recovery (+51.1 points). Despite improving, non-mutants fell behind (+34.6 points), most likely as a result of ongoing restrictive or obstructive pathology. Ratio of FEV1/FVC: In line with healthy lung mechanics (less limitation), mutants returned to 98% normal. The elevated ratio (>107%) maintained by non-mutants was in line with chronic restrictive illness.

5. Conclusion

The findings highlight the necessity of managing TB using a genotype-stratified approach. While non-mutants may need interventions for longterm restriction, mutants may benefit from prolonged anti-inflammatory medications to ameliorate early malfunction. Calls for broader genetic investigations in tuberculosis are echoed by the study's limited generalizability due to its small mutant subgroup (n = 10). Future studies should investigate the mechanistic function of TLR2 in lung regeneration and confirm these findings across a range of populations.

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