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Genetic Polymorphism of *Interferon-Gamma* +874T/A as a Risk Factor for Pulmonary Tuberculosis in Cirebon, Indonesia

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ABSTRACT

Background: Tuberculosis (TB) is one of the leading causes of death globally, caused by *Mycobacterium tuberculosis*. With 10% of all cases worldwide in 2022, Indonesia is the second-largest contributor of tuberculosis cases. *IFN-γ* gene polymorphism is one of the factors that have been studied extensively for its association with TB.

Aims: To analyze *IFN-γ* +874T/A gene polymorphism as a risk factor for pulmonary tuberculosis in Cirebon.

Methods: Observational analysis with case control design was used in this study. Thirty-two tuberculosis patients as cases and 32 healthy controls at RSUD Waled were collected and performed DNA extraction to evaluate the polymorphism by using Amplification-refractory mutation system–polymerase chain reaction (ARMS–PCR). Statistical comparison was performed by using Pearson Chi-square and Kruskal Wallis test. Mann-Whitney U test was done for post hoc test. Odds ratio was calculated to see the risk of the assessed variables, including genotype, allele frequency, and the presence of polymorphism.

Results: In the case group, the frequency of TT genotype was 3 (9.4%), TA genotype was 26 (81.3%), AA genotype was 3 (9.4%). In the control group, the frequency of TT genotype was 12 (37.5%), TA genotype was 17 (53.1%), AA genotype was 3 (9.4%). A significant difference (p=0.034) was found among 3 genotype groups. Post hoc test revealed that TT and TA was the pair with significant difference (p=0.007). In addition, TA polymorphism was significantly associated (p=0.004) with tuberculosis (OR=6.614; CI95% = 1.660-26.349).

Conclusion: *IFN-γ* +874 TA gene polymorphism is associated with pulmonary tuberculosis in the population of Cirebon, West Java, Indonesia.

Keywords: *Polymorphism; Interferon γ; Interferon-gamma; Tuberculosis.*

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1. Introduction

Pulmonary tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, is a serious worldwide health concern that contributes significantly to morbidity and mortality, particularly in nations with low and moderate incomes. This condition primarily affects the lungs, but it can spread to other organs if not treated. TB spreads through airborne droplets, making it extremely contagious, particularly in crowded or poorly ventilated areas. Once ingested, the bacteria can remain latent in the body for years before activating when the immune system is weakened (Geneva: World Health Organization, 2023). Recent study emphasizes the function of the immune system in containing *Mycobacterium tuberculosis* infection, with a focus on cellular responses mediated by T-cells and macrophages that form granulomas to contain the bacteria (Wu et al., 2021). Indonesia is the second country with the largest contribution of tuberculosis cases, accounting for 10% of the global total in 2022. This value is estimated with an incidence rate of around 1,060,000 cases and a mortality rate of around 134,000 (Geneva: World Health organization, 2023). A data from the Indonesian Ministry of Health in 2023 shows the incidence rate of tuberculosis in West Java is estimated at 204,102 confirmed tuberculosis cases. Cirebon Regency is estimated to have 7,202 cases, higher than Cirebon City, which has 2,783 tuberculosis cases (Direktorat Jenderal Pencegahan Kemenkes RI, 2023).

Although TB affects one-third of the world's population, only 10% of those infected actually have the disease. become ill. Most others are infected but do not develop obvious symptoms. Individuals who are infected with tuberculosis and become ill have risk factors, such as diabetes, advanced age, alcoholism, HIV infection, or corticosteroid use. A considerable part of the global population may be naturally immune to tuberculosis infection, which can be influenced by several factors, one of which is genetic factors. Genetic have an important role in pathogenesis. In addition, genetic factors influence an individual's immunological response. (Aravindan PP, 2019; Adane et al., 2021). *Mycobacterium tuberculosis* causes pulmonary tuberculosis, which is heavily impacted by genetic variables in the immune system, including the *interferon-gamma (IFNG/IFN-γ)* gene. The *IFNG* gene produces IFN-γ, a cytokine that activates macrophages and promotes immunological responses against tuberculosis (TB) infection. According to recent research, genetic differences within the *IFNG* gene or its signaling pathways might greatly influence an individual's vulnerability to tuberculosis and the efficacy of immune response, polymorphisms in *IFNG* have been linked to impaired macrophage activation and granuloma formation, which are important defense mechanisms against *Mycobacterium tuberculosis* spread in the lungs. (Aravindan PP, 2019; Adane et al., 2021; Wu et al., 2021).

The SNP (Single Nucleotide Polymorphism) *IFN-γ* +874 T/A, found at the 5'end of the CA repeat in the first intron of human *IFN-γ*, may be linked with tuberculosis. Interferon-gamma (IFN-γ), a cytokine released by activated T cells, natural killer cells, and macrophages, plays an important role in tuberculosis immune response. IFN-γ regulates autophagy and controls intracellular *Mycobacterium tuberculosis* infection (Adane et al., 2021). A study by Adane et al., 2021, found an association between the *IFN-γ* +874 T/A polymorphism and pulmonary tuberculosis among Ethiopian populations. There is no currently research on the *IFN-γ* +874 T/A polymorphism as a risk factor for pulmonary tuberculosis in West Java. This study was conducted to analyze *IFN-γ* +874 T/A polymorphism as a risk factor for pulmonary tuberculosis in Cirebon, West Java.

2. Methods

Study design/ Research procedures

This research includes observational analysis by using case-control design to determine the association between *IFN-γ* T/A polymorphism and the risk for pulmonary tuberculosis. The Ethics Committee of Faculty of Medicine, Universitas Swadaya Gunung Jati has given its approval for this study, with number No.45/EC/FKUGJ/V/2024.

Population and samples

The study group included 32 pulmonary tuberculosis patients and 32 healthy individuals at RSUD Waled. A written consent was taken from the cases and controls subject who met the criteria and the study group was selected using a purposive sampling technique. The sample size of this study was calculated using the sample formula for case and control. Inclusive criteria: (1) Patients diagnosed with pulmonary tuberculosis based on bacteriological results, (2) Patients with drug-sensitive pulmonary tuberculosis, (3) Patients with a history of tuberculosis treatment, (4) Age ≥ 18 years old. Exclusion criteria: (1) Patients with comorbid (Diabetes Mellitus (DM), HIV/AIDS), (2) Patients who consumes an alcohol, (3) Patients who consumes long term immunosuppressant drugs. Healthy controls: Individuals aged ≥ 18 years old who had never been diagnosed with pulmonary tuberculosis and had no symptoms of pulmonary tuberculosis measured by questionnaire completed by physician to ensure data input done correctly, and had never been in close contact with pulmonary tuberculosis patients.

Measurements

The *IFN-γ* +874 T/A polymorphism is the study's independent variable and the dependent variable is the pulmonary tuberculosis. The pulmonary tuberculosis data was taken using secondary data from patient's medical records and screening form. The polymorphism was observed by using ARMS–PCR (Amplification-refractory mutation system–polymerase chain reaction).

Genotyping

ARMS PCR was performed to genotype each gene, with specific primers. The primer sequencing was as follows: Sense *IFN-γ* (+874) A:5'-TTCTTACAACACAAAATCAAATCA-3', Sense *IFN-γ* (+874) T : 5' TTCTTACAACACAAAATCAAATCT-3', Antisense *IFN-γ* (+874) : 5'-TCAACAAAGCTGATACTCCA-3' (Adane et al., 2021). The PCR reaction was performed in total volume 25 µl, containing Forward Primer 1 µl, Reverse Primer 1 µl, Go Taq Polymerase enzyme 12,5 µl, DNA template 3 µl, H2O 7,5 µl. The PCR amplification was performed using 35 cycles (denaturation 95◦C for 5 min., annealing 53◦C for 1 min., extension 68◦C to 72◦C for 1 min.). PCR products were examined by electrophoresis with 1.5% agarose gel and gel red 1 µl.

Statistical techniques

Univariate and bivariate analysis were used to statistically examine the data. The Chi-square test was used to asses demographics characteristic and allele frequencies. The Kruskal Wallis test was used to assess genotype frequencies, and Post-hoc analysis was performed using the Mann-Whitney U test. To compare the genotype frequencies of two, the Chi-square test was used. For the 95% Confidence Interval (95%CI) and odds ratio (OR), a P-value of less than 0.05 was considered significant.

3. Results

Respondent characteristics

In this study, 32 healthy controls and 32 pulmonary tuberculosis patients participated. Table 1 summarizes the respondent characteristics in this study including age, gender, and smoking history. The majority of patients with TB case were female (62.5%) while in the control groups the majority of patients were male (65.6%), there was a difference in sex distribution of both populations (P=0.024). There were no differences found in the age and history of smoking distributions in the two study groups.

Table 1. Respondents' Characteristics

Characteristics	Case n(%)	Control n(%)	P-value
Total	32(100.0)	32(100.0)	
Age			
18-45	11(34.4)	11(34.4)	
46-59	10(31.3)	15(46.9)	0.291[a]
≥60	11(34.4)	6(18.8)	
Gender			
Male	12(37.5)	21(65.6)	0.024[a]*
Female	20(62.5)	11(34.4)	
Smoking			
Yes	5(15.6)	10(31.3)	0.140[a]
No	27(84.4)	22(68.8)	

[a]P-value were calculated by the Chi-square test

Genotyping of IFN-γ +874T/A

A 261bp DNA fragment was identified by ARMS-PCR using specific primers for the A and T alleles (Fig. 1). The frequency of T allele was higher in control group (64.1%), while the A allele was higher in the case group (51.6%). In both the case and controls groups, TA was the most frequent genotype, in the case group TA was higher (81.3%) than in the control group (53.1%). In the control group the TT (37.5%) genotype was more common than AA (9.4%) genotype. The genotype distribution showed significant difference (p=0.034). Post hoc test revealed that TT and TA was the pair with significant difference (p=0.007). (Table 2).

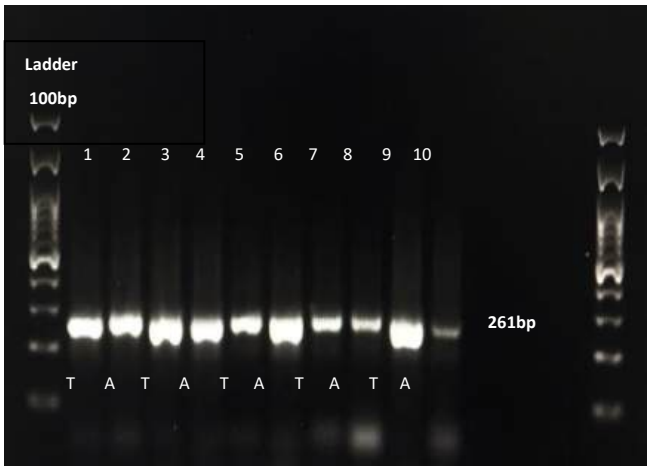

Figure 1. : Results from gel electrophoresis with marker. Lanes 1, 3, 5, 7, 9 shows the T allele (261bp). Lanes 2, 4, 6, 8, 10 shows allele A (261bp).

Table 2. Frequencies of genotypes and allele

Genotype	Case	Control	P-value
	n(%)	n(%)	
TT	3(9.4)	12(37.5)	
TA	26(81.3)	17(53.1)	0.034[a]
AA	3(9.4)	3(9.4)	
Allele			
T	31(48.4)	41(64.1)	0.075[b]
A	33(51.6)	23(35.9)	

[a]P-value were analyzed by the Kruskal Wallis, [b]P-value were analyzed by the Chi-square test

Relationship between *IFN-γ* +874T/A gene polymorphism with Pulmonary Tuberculosis were showed in Table 3. There was a significant association between polymorphism (p=0.004) and tuberculosis (OR=6.614; CI95% = 1.660-26.349).

Table 3. Relationship between *IFN-γ* +874T/A gene polymorphism with Pulmonary Tuberculosis

Polymorphism	Case	Control	P-value	OR (95%CI)
	n(%)	n(%)		
Yes (TA-AA)	29(90.6)	19(59.4)	0.004[a]	6.614(1.660-26.349)
No (TT)	3(9.4)	13(40.6)		

[a]P-value were calculated by the Chi-Square test

4. Discussion

Mycobacterium tuberculosis, the bacteria that causes pulmonary tuberculosis, is one of the main causes of morbidity and death worldwide (Geneva: World Health organization, 2023). There are risk factors that can affect tuberculosis patients, such as sociodemographic factors, environmental factors, and host-related factors, one of which is genetic factors that can affect tuberculosis immunity.

In this research, there was a significant sex difference between the case and control groups, these findings are in line with a study conducted by Nguyen HV, et al., 2023, in a Vietnamese population, there was a significant difference in the prevalence of tuberculosis related to sex, with behavioral and environmental risks, sex determining the behavioral and environmental risks that lead to tuberculosis. Some studies suggest that the high male:female ratio is mainly due to underreporting of female cases and reporting bias, social roles affect how people interact with those who have *Mycobacterium tuberculosis*, how men and women seek and access tuberculosis services, and how some male-dominated occupations (like mine workers) may raise the risk of tuberculosis (Nguyen et al., 2023).

Genetic factors have an important role in pathogenesis. In addition, genetic factors influence an individual's immunological response, Polymorphism in different genes can impact and alter immune response which is why not all *Mycobacterium tuberculosis* infections lead to tuberculosis illness (Aravindan PP, 2019). Mutations in the *IFN-γ* gene increase risk of developing bacterial, viral, or parasitic infections as well as a number of autoimmune disorders. Genetic variety is the basis of human diversity and there are many genes encode different cytokines that play important roles in host susceptibility to tuberculosis. IFN-γ is one of the important cytokines in tuberculosis immunity, generated by activated T cells, natural killer cells, and macrophages. IFN-γ production activates macrophages and promoting cell proliferation, adhesion and apoptosis. IFN-γ induces a large number of Reactive Oxygen Intermediates (ROI) and nitric oxide production to control *Mycobacterium tuberculosis* infection (Desai, 2022). The identification of genetic factors that influence different stages and forms of tuberculosis is crucial to understanding its pathophysiology. These findings should have significant implications for tuberculosis control, including improved prevention techniques, vaccine optimization, and clinical trials and the development of new treatments focused at restoring inadequate immune responses (Abel, El-Baghdadi, Bousfiha, Casanova, & Schurr, 2014)

Research has focused on the association between *IFN-γ* +874 T/A polymorphisms and tuberculosis susceptibility, more studies have been conducted on specific single nucleotide polymorphisms (SNPs) in the promoter region of the *IFN* gene, including variants +874T/A, -1616T/C, and +874A/T (Wu et al., 2021; Cai et al., 2019). In this study, the frequency distribution of polymorphisms in the *IFN-γ* +874 T/A was obtained through ARMS-PCR examination. The reference genotype = TT, while the polymorphisms are TA and AA genotypes. This study showed that the majority of subjects in the case group (90.7%) and control group (62.5%) had *IFN-γ* +874 T/A polymorphism. The results of the calculation of the odds ratio of polymorphism obtained a value of 6.614, meaning that someone with polymorphism has a risk of 6.614 times more susceptible to having pulmonary tuberculosis disease compared to someone who does not have polymorphism. There is a statistically significant association between *IFN-γ* +874 T/A polymorphism and the risk of pulmonary tuberculosis disease.

The significant association between *IFN-γ* +874 T/A polymorphism and the risk of pulmonary tuberculosis disease of this study are in line with cross sectional research conducted by Adane et al., 2021, in Ethiopia involving 200 people found that there is a significant association between *IFN-γ* +874 T/A gene polymorphism and pulmonary tuberculosis disease. Another study conducted by et al., 2018, with the case control method in

the Iranian population, a study involving 195 people found that the *IFN-γ* +874T/A gene polymorphism was significantly associated with susceptibility to tuberculosis. Research conducted by Wu et al., 2021 in the Chinese population involving 201 people found that the *IFN-γ* +874T/A gene polymorphism was susceptible to tuberculosis. This mechanism may be related to the AA genotype patients are more susceptible to experience a Th1/Th2 imbalance. Due to weakened immune systems, they may be more vulnerable to *Mycobacterial* infections that cause tuberculosis. Different results were shown by a study in Medan, Indonesia conducted by Kaban, et al., 2021 which showed the *IFN-γ* +874 T/A gene polymorphism was not significantly associated with susceptibility to pulmonary tuberculosis. The limitations of the study should be highlighted, such a small sample size of the study. This study did not assess other risk factors for pulmonary tuberculosis that lead to immunocompromised conditions such as a history of DM, HIV, and long-term immunosuppressant drugs use.

5. Conclusion

In conclusion, *IFN-γ* +874 TA polymorphism is associated with pulmonary tuberculosis in the population of Cirebon, West Java, Indonesia. However, larger sample research in other population groups is suggested.

Conflict of Interest

The author declares there is no conflict of interest.

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