Original Article

Association of IL – 23 R rs 7518660 Gene Polymorphism with Susceptibility and Disease Severity of Pulmonary Tuberculosis

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ABSTRACT

Pulmonary Tuberculosis (TB) is a global health problem. Of all people infected with Mycobacterium tuberculosis only a small proportion develops into TB. IL 23 is the key cytokine in the pathogenesis of TB infection. This study aims to determine the association of IL-23 R rs 7518660 gene polymorphism with susceptibility and disease severity of Pulmonary TB. A case control study involved 105 people consisting of 31 drug sensitive pulmonary TB patients, 40 patients with drug-resistant pulmonary TB and 34 healthy subjects as a control. IL-23 R rs 7518660 gene polymorphism G allele increases susceptibility to both TB drug-sensitive and drug-resistant. G and A allele, AA and AG genotypes indicates (p value >0.05) in correlation with disease severity based on lesion in chest X-ray and high load of Mycobacterium tuberculosis in sputum. There was a significant relationship between allele A and susceptibility to pulmonary TB with an odds ratio of 0.231. It showed that patients with A alleles (AG and AA genotypes) were at risk of developing TB by 1/0.231 = 4.33 times lower than patients with GG genotypes. Meanwhile, the relationship of the G allele with susceptibility to pulmonary TB obtained (p value <0.05) and an odds ratio value of 0.127 indicating that patients with G alleles (GG and AG genotypes) were at risk of developing TB of 1/0.127 = 7.87 times higher than in patients with the AA genotype. Conclusion: We found significant correlation between IL-23 R rs 7518660 gene polymorphism G allele with susceptibility to pulmonary TB, but the result was not significant with disease severity.

Keywords: disease severity; IL-23 R rs 7518660 gene; polymorphism; pulmonary TB; susceptibility,

ABSTRAK

Tuberkulosis (TB) Paru merupakan masalah kesehatan global. Dari semua orang yang terinfeksi Mycobacterium tuberculosis hanya sebagian kecil yang berkembang menjadi TB. IL23 adalah sitokin kunci dalam patogenesis infeksi TB. Penelitian ini bertujuan untuk mengetahui hubungan polimorfisme gen IL-23 R rs 7518660 dengan kerentanan dan keparahan penyakit TB Paru. Studi kasus kontrol melibatkan 105 orang yang terdiri dari 31 pasien TB paru sensitif obat, 40 pasien TB paru resisten obat dan 34 subjek sehat sebagai kontrol. Polimorfisme gen IL-2.3 R rs 7518660 alel G meningkatkan kerentanan terhadap TB sensitif obat dan resisten obat. Alel G dan A, genotipe AA dan AG menunjukkan (p value >0.05) berkorelasi dengan derajat keparahan penyakit berdasarkan lesi pada rontgen dada dan tingginya kadar Mycobacterium tuberculosis dalam sputum. Terdapat hubungan yang bermakna antara alel A dengan kerentanan terhadap TB paru dengan odds ratio sebesar 0,231. Hal ini menunjukkan bahwa pasien dengan alel A (genotipe AG dan AA) berisiko terkena TB sebesar 1/0.231 = 4.33 kali lebih rendah dibandingkan pasien dengan genotipe GG. Sedangkan uji hubungan alel G dengan kerentanan terhadap TB paru diperoleh (p<0.05) dan nilai Odd ratio 0.27 yang menunjukkan bahwa pasien dengan alel G (genotipe GG dan AG) berisiko mengalami TB 1/0,127 = 7.87 kali lebih tinggi

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dibandingkan pada pasien dengan genotipe AA. Terdapat korelasi yang signifikan antara polimorfisme gen IL-23 R rs 7518660 alel G dengan kerentanan terhadap TB paru, tetapi tidak signifikan dengan keparahan penyakit.

Kata kunci: gen IL-23 R rs 7518660; kerentanan; keparahan penyakit; polimorfisme; TB paru

INTRODUCTION

Tuberculosis (TB) is an infectious disease that is currently the main cause of health problems. About a quarter of the world's population are infected with *Mycobacterium tuberculosis*. The biggest contributors to the global increase in TB worldwide are from India and Indonesia. In India, people newly diagnosed with TB increased by 1.2 million to 2.2 million between 2013 and 2019 (74%). In Indonesia, the number increased from 331,703 in 2015 to 562,049 in 2019 (69%). The target of the 2030 Sustainable Development Goals (SDGs) is to reduce TB mortality by 90% and reduce TB incidence by 80%.[1]

Of all people infected with *Mycobacterium tuberculosis*, only about 5-10% become sick; this is related to the body's immune response at the beginning of *Mycobacterium tuberculosis* infection[4]. A person's susceptibility to TB is determined by genetic factors encoded in genes in Deoxyribose Nucleic Acid (DNA) molecular strand, in which the distribution is different for each population and race. The role of gene polymorphism will change the structure of the protein produced so that it will affect individual phenotypes, including susceptibility to disease.[3, 5]

Drug-sensitive TB is a form of TB that is still sensitive to first line Anti Tuberculosis Drugs. This condition requires fast, precise, and directed treatment and action thus TB patients do not develop to the-drug resistance stage. As many as 96% of cases of resistance to Rifampicin are caused by mutations in the 'hot-spot region' by 81 bp spanning codons 507-533 in the rpoB gene.[6] According to the World Health Organization (WHO) in 2017, incidence of Multi Drug Resistance (MDR) TB cases amounted to around 3.3% of all new cases, and overall patients had received Anti Tuberculosis Drugs therapy previously (20%). Data obtained from dr. Saiful Anwar Hospital Malang reached 21 new patients every month.[7]

Several pro-inflammatory cytokine variants are associated with the possibility of pulmonary TB, one of them is the IL-23 R rs 7518660 gene polymorphism. IL-23 plays a role in the regulation of the immune system in the TB infection process[15]. Based on the data above, this study analyzes the presence of the IL-23 R rs 7518660 gene polymorphism which is associated with the susceptibility and severity of pulmonary TB in patients.[9,11]

MATERIALS AND METHODS

Research Design

This research used a Case-Control Study design. The aim of this study was to determine IL-23 R rs 7518660 gene polymorphism with pulmonary TB by comparing the case and the control group based on their exposure status.

Research Subjects and Sample Size

The sample population were patients with pulmonary TB who seek treatment at the outpatient clinic of dr. Saiful Anwar Hospital Malang. All ethnic groups who seek treatment at the Pulmonary Clinic or being hospitalized at dr. Saiful Anwar Hospital Malang were included in the study and recorded.

Case group: Patients with drug-sensitive and drug-resistant pulmonary TB.

Control group: Healthy subjects.

Inclusion and Exclusion Criteria
Inclusion Criteria:
a. Patients diagnosed with drug-sensitive or drug-resistant pulmonary TB
b. Age between 18 – 65 years old
c. Willing to participate in research and sign the "informed consent"

Exclusion Criteria:
a. Patients with HIV-AIDS
b. Patients with autoimmune disease
c. Pregnant women
Note: Patients with Diabetes Mellitus, Chronic Kidney Disease, malnutrition, and smoking were not excluded in this research, but they were still given notes for data analysis (information is listed in Table 1)

Research Variables
Independent Variable:
a. IL-23 R rs 7518660 gene polymorphism
Dependent Variables:
a. Susceptibility to drug-sensitive and drug-resistant pulmonary TB
b. The severity of pulmonary TB, based on chest X-ray lesion and the number of *Mycobacterium tuberculosis* detected on GeneXpert sputum.

From chest X-ray lesion, the patients with minimal and moderate lesion added categorized as mild criteria, whereas the far advanced lesion added into severe criteria. From the data of GeneXpert sputum, we divided into two categories regarding to the number of thresholds based on repeat cycles of *Mycobacterium tuberculosis* DNA amplification. Very low and low added were categorized as mild criteria, whereas medium and high added into severe criteria.

Data Collection
Samples were obtained by consecutive sampling method in patients who met the inclusion and exclusion criteria in the outpatient and hospitalized patients at dr. Saiful Anwar Hospital Malang.

IL-23 R rs 7518660 Gene Polymorphism Examination Procedure
Identification of the allele position where the polymorphism occurred was performed by incubating the Polymerase Chain Reaction product at 94ºC for 30 seconds to denature the DNA genome, followed by primer annealing at 68ºC for 20 seconds and extension at 72ºC for 20 seconds.[10,14] Polymerase Chain Reaction was performed for 35 cycles, followed by a final extension at 72ºC for three minutes. Each sample is grouped according to the results of 2% agarose gel electrophoresis, while the visualization of the gel electrophoresis results were performed using a UV-transilluminator and a polaroid camera.[9,10,14]

Data Processing and Analysis Techniques
Processing and data analysis were performed with IBM SPSS software version 26.0. The relationship between polymorphism with susceptibility and severity of pulmonary TB was analyzed using the Chi-square test using a 95% confidence level, significant if $p<0.05$. Meanwhile, to determine the magnitude of the risk factor, it was calculated using the odds ratio (OR).

RESULTS AND DISCUSSION

Sociodemographic Characteristics of Research Subjects
This research was conducted on 105 samples which were divided into three groups. The healthy control group consisted of 34 samples and the TB case group consisted of 71 samples. TB cases were composed of 31 samples of drug-sensitive TB and 40 samples of drug-resistant TB. The sociodemographic characteristics of the research subjects can be seen in Table 1.

Table 1. Sociodemographic Characteristics of Research Subjects

Characteristics		Healthy control (n=34)	TB Cases		p-value
			Drug sensitive (n=31)	Drug resistant (n=40)	
Age	Minimum	29	19	18	0.001[a]
	Maximum	58	69	69	
	Mean ± SD	33.79 ± 4.98	40.42 ± 14.09	45.5 ± 13.06	
Gender	Male	20 (58.8%)	15 (48.4%)	24 (60%)	0.577[b]
	Female	14 (41.2%)	16 (51.6%)	16 (40%)	
Body Mass Index (BMI)	Minimum	19.3	13.8	12.1	0.000[a]
	Maximum	31.1	29.9	26.1	
	Mean ± SD	22.86 ± 2.86	18.55 ± 3.89	18.5 ± 3.04	
Smoking status	Yes	1 (2.9%)	4 (12.9%)	4 (10%)	0.329[b]
	No	33 (97.1%)	27 (87.1%)	36 (90%)	
Diabetes Mellitus (DM)	Yes	-	8 (25.8%)	13 (32.5%)	0.540[b]
	No	-	23 (74.2%)	27 (67.5%)	
Chronic Kidney Disease (CKD)	Yes	-	2 (6.5%)	1 (2.5%)	0.412[b]
	No	-	29 (93.5%)	39 (97.5%)	
Malnutrition	Yes	-	21 (67.7%)	21 (52.5%)	0.195[b]
	No	-	10 (32.3%)	19 (47.5%)	

a: Kruskal Wallis test　　　　　　　　　　　　　　　(Source: Primary Research Data Processed)
b: Chi-square test
SD: Standard Deviation

Based on the characteristics of the research subjects, the average age of the healthy control group was 33.79 ± 4.98 years old, the drug-sensitive TB group was 40.42 ± 14.09 years old, and the drug-resistant TB group was 45.5 ± 13.06 years old. For the age variable, a normality test was performed using the Shapiro-Wilk test. The research variable was normal if the p-value > 0.05. The result of the normality test for the age variable was p-value=0.000, which showed that the normality of the data was not met for this variable. Furthermore, the Kruskal Wallis test was performed and obtained a p-value of 0.001 (p<0.05) which proved that there was a significant difference in age characteristics in the three groups, where the healthy control group had a lower average age than the TB case group.

In terms of gender characteristics, the Chi-square test was performed and obtained a p-value of 0.577 (p>0.05) which proved that

there was no gender difference between the three groups.

In terms of Body Mass Index (BMI) characteristics, the average BMI of healthy control group was 22.86 ± 2.86, the TB SO group was 18.55 ± 3.89 and the TB RO group was 18.5 ± 3.04. By using the Kruskal-Wallis test, a p-value of 0.000 (p<0.05) was obtained which proved that there was a significant difference in the characteristics of BMI in the three groups, where the TB group, both drug-sensitive and drug-resistant, had a lower average BMI compared to the healthy control group.

Based on the smoking status and the comorbid characteristics of TB patients, such as Diabetes Mellitus, Chronic Kidney Disease and malnutrition, p-value more than 0.05 (p>0.05) was obtained. From this test, it showed that there were no significant differences in smoking status and the comorbid characteristics of TB patients.

Clinical Characteristics of Research Subjects

Based on the imaging of the chest X-ray lesion in Figure 1, it showed that the most common lesion in the TB case group, both in the drug-sensitive and drug-resistant TB groups, were more (far) advanced lesion as much as 81.69%. The minimal lesion was the least chest X-ray imaging (1.41%). The following charts describe the chest X-ray lesion in each case groups.
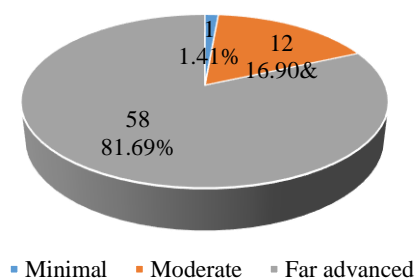

Figure 1. Description of Chest X-ray Lesion in Each Case Group

The imaging of chest X-ray lesion in each group of TB cases is presented in Table 2. Based on Table 2, it shows that, in the drug-sensitive TB group, the most extensive or far advanced lesion description was 87.1%. Likewise, in the drug- resistant TB group, the most extensive lesion description was 77.5%. By using the Chi-square test, a p-value of 0.474 (p>0.05) was obtained. From this test, it showed that there was no significant difference in chest X-ray images between the drug-sensitive TB and drug-resistant TB groups.

Table 2. The Imaging of the Chest X-ray Lesion in the Case Group

The Imaging of Chest X-ray Lesion	Groups		p-value
	Drug-sensitive TB	Drug-resistant TB	
Minimal	0 (0%)	1 (2.5%)	
Moderate	4 (12.9%)	8 (20%)	0.474
Far advanced	27 (87.1%)	31 (77.5%)	

(Source: Primary Research Data Processed)

Based on the number of *Mycobacterium tuberculosis* detected in sputum, Figure 2 shows that the number of *Mycobacterium tuberculosis* in the GeneXpert sputum was mostly in TB case group, both in the drug-sensitive and drug-resistant TB groups were at a medium level of 42.25%. The very low level was a picture of the least number of GeneXpert sputum examination, which was 7.04%. The following chart describes the number of *Mycobacterium tuberculosis* detected on sputum examination in each case group.
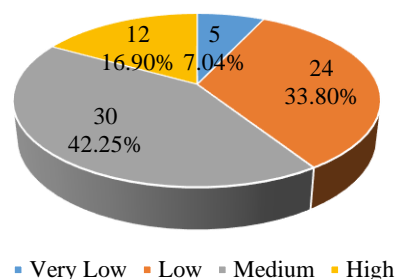

Figure 2. Overview of the GeneXpert Sputum of the *Mycobacterium tuberculosis* Case Group

Based on the number of *Mycobacterium tuberculosis* detected in sputum, Figure 2 shows that the number of *Mycobacterium tuberculosis* in the GeneXpert sputum was mostly in TB case group, both in the drug-sensitive and drug-resistant TB groups were at a medium level of 42.25%. The very low level was a picture of the least number of GeneXpert sputum examination, which was 7.04%. The following chart describes the number of *Mycobacterium tuberculosis* detected on sputum examination in each case group.

The description results of the number of *Mycobacterium tuberculosis* detected in the GeneXpert sputum is presented in Table 3. Based on Table 3 shown in the drug-sensitive TB group, the number of *Mycobacterium tuberculosis* detected in the GeneXpert sputum was mostly in the low category, which was 58.1%. Meanwhile, in the drug-resistant TB group, the GeneXpert sputum description was mostly in the medium category, which was 50%. By using the Chi-square test, a p-value of 0.001 (p<0.05) was obtained. From this test, it showed that there were significant differences in the results of the GeneXpert sputum between the drug-sensitive TB and drug-resistant TB groups.

Table 3. Overview of the Number of *Mycobacterium tuberculosis* Detected on GeneXpert Sputum in Research Subjects

Number of Mycobacterium Tuberculosis Detected on GeneXpert Sputum	Groups		p-value
	Drug-sensitive TB	Drug-resistant TB	
Very Low	1 (3.2%)	4 (10%)	
Low	18 (58.1%)	6 (15%)	0.001
Medium	10 (32.3%)	20 (50%)	
High	2 (6.5%)	10 (25%)	

(Source: Primary Research Data Processed

The distribution of patient types is described in Figure 3 which shows that the most drug-resistant TB patient types were relapse and new cases, each group consisted of 14 people (35%). The lowest type of TB drug-resistant patient was K1 failure.
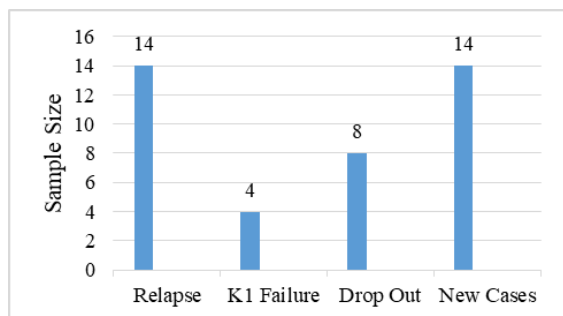
Figure 3. Description of the Distribution of the Type of TB Patients RO

The allele frequencies and genotypes of the IL-23 R rs 7518660 gene polymorphism in the healthy and TB control groups, on both drug-sensitive and drug-resistant TB are presented in Table 4.

Based on Table 4, it is shown that the frequency of the G allele in the control group was 17 (50%), the drug-sensitive TB group was 30 (96.8%) and the drug-resistant TB group was 33 (82.5%). The frequency of the G allele was more in the TB case group than in the control group. From the Chi-square test results obtained p-values of 0.000 (K vs drug-sensitive TB) and 0.003 (K vs drug-resistant TB).

From this test, it was proven that there was significant difference in the frequency of the G allele between the TB group and the control group. Meanwhile, the comparison of the G allele frequency between the drug-sensitive TB and drug-resistant TB groups obtained a p-value of 0.059 (p>0.05), which showed that there was no significant difference in the frequency of the G allele.

Table 4. Comparison of the Allele Frequencies and Genotypes of IL-23 R rs 7518660 Gene Polymorphism in TB and Healthy Controls

Variables	Healthy control (K) (n=34)	TB Cases		p-value		
		Drug-sensitive TB (n=31)	Drug-resistant TB (n=40)	K vs drug-sensitive TB	K vs Drug-resistant TB	Drug-sensitive vs Drug-resistant TB
GG	4 (11.8%)	9 (29%)	17 (42.5%)	0.082^{ns}	0.003	0.243^{ns}
AG	13 (38.2%)	21 (67.7%)	16 (40%)	0.017	0.877^{ns}	0.020
AA	17 (50%)	1 (3.2%)	7 (17.5%)	0.000	0.003	0.059^{ns}
Alel G	17 (50%)	30 (96.8%)	33 (82.5%)	0.000	0.003	0.059^{ns}
Alel A	30 (88.2%)	22 (71%)	23 (57.5%)	0.082^{ns}	0.003	0.243^{ns}

ns: Not Significant   (Source: Primary Research Data Processed)

Table 5 below describes the results of analysis of the relationship between the IL-23 R rs 7518660 gene polymorphism with susceptibility of pulmonary TB. GG genotype was used as a comparison.

Table 5. Analysis of the Relationship Between the IL-23 R rs 7518660 Gene Polymorphism with the Susceptibility of Pulmonary TB

Variables		Healthy Control	Drug-sensitive and Drug-resistant TB	p-value	OR	95% CI
Genotype	GG	4 (11.8%)	26 (36.6%)		(Reff)	
	AG	13 (38.2%)	37 (52.1%)	0.180^{ns}	0.438	0.128 - 1.495
	AA	17 (50%)	8 (11.3%)	0.000*	0.072	0.019 - 0.278
Allele G		17 (50%)	63 (88.7%)	0.000*	0.127	0.047 - 0.344
Allele A		30 (88.2%)	45 (63.4%)	0.008*	0.231	0.073 - 0.729

ns: Not Significant   (Source: Primary Research Data Processed)
*: Significant

The relationship between the AG genotype and susceptibility to pulmonary TB obtained a p-value of 0.180 which proves that there was no significant relationship between the AG genotype and susceptibility to pulmonary TB (OR = 0.438 (0.128 - 1.495)). While the AA genotype obtained a p-value of 0.000 which proves that there was a significant relationship between the AA genotype and susceptibility to pulmonary TB. The odds ratio of 0.072 (0.019 - 0.278) indicates that patients with the GG genotype are at risk of developing TB by 1/0.072 = 13.81 times higher than patients with the AA genotype.

Table 6. Analysis of the Relationship Between the IL-23 R rs 7518660 Gene Polymorphism with the Severity of Pulmonary TB Based on Chest X-ray Lesion

Variables		Minimal and Moderate Lesion	Far Advanced Lesion	p-value	OR	95% CI
Genotype	GG	4 (30.8%)	22 (37.9%)		(Reff)	
	AG	6 (46.2%)	31 (53.4%)	0.929	0.939	0.237 - 3.727
	AA	3 (23.1%)	5 (8.6%)	0.176	0.303	0.051 - 1.805
Allele G		10 (76.9%)	53 (91.4%)	0.136	0.314	0.065 - 1.531
Allele A		9 (69.2%)	36 (62.1%)	0.628	0.727	0.200 - 2.647

(Source: Primary Research Data Processed)

Table 6 describes the results of the relationship analysis between IL-23R rs 7518660 gene polymorphism and the severity of pulmonary TB based on Chest X-ray lesion where the GG genotype was used as a comparison. The relationship between the AG genotype and the severity of pulmonary TB obtained a p-value of 0.929, which proves that there was no significant relationship between the AG genotype and the severity of pulmonary TB (OR = 0.939 (0.237 - 3.727). Likewise, the AA genotype showed no significant relationship between the AA genotype and the severity of pulmonary TB (p>0.05).

Table 7. Analysis of the relationship between the IL-23R rs 7518660 gene polymorphism with the severity of pulmonary TB based on the number of *Mycobacterium tuberculosis* detected on GeneXpert sputum

Variables		Very Low and Low	Medium and High	p-value	OR	95% CI
Genotype	GG	4 (30.8%)	22 (37.9%)		(Reff)	
	AG	6 (46.2%)	31 (53.4%)	0.337	1.643	0.595 - 4.538
	AA	3 (23.1%)	5 (8.6%)	0.213	3.000	0.508 - 17.708
Allele G		27 (93.1%)	36 (85.7%)	0.333	2.250	0.421 - 12.028
Allele A		16 (55.2%)	29 (69%)	0.233	1.813	0.679 - 4.837

(Source: Primary Research Data Processed)

Table 7 describes the results of relationship analysis between the IL-23 R rs 7518660 gene polymorphism and the severity of pulmonary TB based on the number of *Mycobacterium tuberculosis* detected in GeneXpert sputum where the GG genotype was used as a comparison. The relationship between the AG genotype and the severity of pulmonary TB obtained a p-value of 0.337, which proves that there was no significant relationship between the AG genotype and the severity of pulmonary TB (OR = 1.643 (0.595 - 4.538)). Likewise, the AA genotype showed no significant relationship between the AA genotype and the severity of pulmonary TB ($p>0.05$).

In the results of relationship analysis between the G allele and the severity of pulmonary TB based on the number of *Mycobacterium tuberculosis* detected in GeneXpert sputum, a p-value of 0.333 ($p>0.05$) was obtained, which proves that there was no significant relationship between the G allele and the severity of pulmonary TB (OR = 2.250 (0.421 - 12.028)). Likewise, the results of testing the relationship between the A allele and the severity of pulmonary TB showed that there was no significant relationship between the A allele and the severity of pulmonary TB ($p>0.05$).

For age characteristics, a p-value of 0.001 ($p<0.05$) was obtained which proves that there was a significant difference in age characteristics in the healthy control group, drug-sensitive TB and drug-resistant TB, where the healthy control group has a lower average age than the TB case group.[17] In this study, the average age in the drug-sensitive and drug-resistant TB groups was the productive adult age group, but the drug-sensitive TB group was slightly younger than the drug-resistant TB group[13]. Based on data from the WHO in 2015 and Permenkes 2016, it was stated that the incidence of pulmonary TB was highest in the productive adult age group. Research from Dodd et al. stated that the incidence of pulmonary TB in adults was 1.5-6 times higher than in children and adolescents. This is due to the tendency of greater social interaction in adulthood. [22]

The clinical characteristics of the subjects of this study used the parameters of the lesion area on the chest X-ray and the number of *Mycobacterium tuberculosis* detected in the GeneXpert examination to be assessed based on the severity of pulmonary TB. The results showed that, in the drug-sensitive TB group and drug-resistant TB group, the most extensive or far advanced lesion were 87.1% and 77.5%, respectively. Based on the correlation test of the X-ray lesion area parameters, it showed that there was no difference in the imaging of the chest X-ray lesion between the drug-sensitive TB and drug-resistant TB groups. Icksan et al.[23] stated that the most common lesion on the chest X-ray was extensive lesion consisting of cavities, consolidation, fibrosis with atelectasis, bullae, and calcifications where the degree of damage was more extensive in the drug-resistant TB group.[23]

Based on the results of the number of *Mycobacterium tuberculosis* detected in the GeneXpert sputum examination, it was

shown that there were differences in the GeneXpert sputum examination between the drug-sensitive TB and drug-resistant TB groups. In the drug-sensitive TB group, the most *Mycobacterium tuberculosis* detected was in low level (58.1%), while in the drug-resistant TB group, *Mycobacterium tuberculosis* detected was in medium level (50%). GeneXpert examination in the drug-resistant TB group showed more *Mycobacterium tuberculosis* than the drug-sensitive TB group. The number of *Mycobacterium tuberculosis* detected on the GeneXpert examination was directly proportional to the viscosity of the sputum examined. Our study used the GeneXpert sputum examination which has a more sensitive result than the Acid Fast Bacillus (AFB) examination[8]. The analysis of sputum samples used the Polymerase Chain Reaction method which calculates the number of thresholds based on repeat cycles of *Mycobacterium tuberculosis* DNA amplification. The GeneXpert method is semi-quantitative, while the high level is stated if there is 16 *Mycobacterium tuberculosis* Ct, medium if there is 16-22 *Mycobacterium tuberculosis* Ct, low if there is 22-28 *Mycobacterium tuberculosis* Ct, and very low if there is 28-38 *Mycobacterium tuberculosis* Ct. Therefore, the lower the *Mycobacterium tuberculosis* Ct number, the higher *Mycobacterium tuberculosis* number that will be detected.[23,24,26]

In this study, the frequency of IL-23 R rs 7518660 G allele polymorphism and the AG genotype were higher in the pulmonary TB group than in healthy controls and it was statistically significant. This was on concordance with the results of previous research on the Chinese Uygurs ethnic group in 2015 which stated that the allele frequencies studied showed significant differences between the case group and healthy controls, where the G allele was more dominant in the TB case group compared to healthy controls. Jiang et al.'s[9] study for the AG genotype with an odds ratio of 2.99 had 0.34 times less chance of developing TB than

the GG genotype. Based on statistical data analysis, the frequency of IL-23R rs 7518660 gene polymorphism genotype AA was higher in healthy controls. This indicates the protective effect of allele A against pulmonary TB. Research in Tunisia in 2012 found that the frequency of the A allele and the AA genotype increased the risk 2.79 times greater for the incidence of pulmonary TB.[9,18]

In this study, there was a significant relationship between allele A and susceptibility to pulmonary TB with an odds ratio of 0.231. This showed that patients with A alleles (AG and AA genotypes) were at risk of developing TB by 1/0.231 = 4.33 times lower than patients with GG genotypes. Meanwhile, the relationship test of the G allele with susceptibility to pulmonary TB obtained a p-value of 0.000 (p<0.05) and an odds ratio value of 0.127, indicating that patients with G alleles (GG and AG genotypes) were at risk of developing TB of 1/0.127 = 7.87 times higher than in patients with the AA genotype. This also in concordance with the research conducted on the Chinese Uygur ethnic group in 2015, which stated that a person with the G allele has a higher risk of developing pulmonary TB compared to the A allele with an odds ratio of 4.83.[9]

Colonization of *Mycobacterium tuberculosis* caused widespread organ damage and was at risk of mutation[12]. The results showed that there was no significant relationship between the A, G alleles and the AA, AG genotype with the severity of pulmonary TB based on the lesion on the chest X-ray (p>0.05). Previous studies on Chinese Uygurs also did not show significant results on the severity of lesion on chest radiographs. The same study examined the polymorphism of the IL-23 R SNP gene rs1884444 which showed a significant relationship to the severity of pulmonary TB based on chest X-ray lesion.[19]

In this study, we analyzed whether there was a relationship between the IL-23 R rs 7518660 gene polymorphism and the severity

of pulmonary TB based on the number of *Mycobacterium tuberculosis* detected in GeneXpert sputum. The results showed that there was no significant relationship between the A, G alleles and the AA, AG genotype with the severity of pulmonary TB based on the number of *Mycobacterium tuberculosis* detected in GeneXpert sputum (p>0.05). Shabbir et al. (2007) stated that one of the influencing factors was the limitation in checking the quality of sputum samples. It is known that the good sputum quality will result in a good sampling process, which that will determine the number of bacteria, which will subsequently determine the level of transmission and the severity of TB patients.[20,21]

The absence of a significant relationship between the IL-23 R rs 7518660 gene polymorphism in terms of both alleles and genotypes on the severity of TB based on the number of *Mycobacterium tuberculosis* detected on sputum examination was also shown by a study conducted in the Uygurs of China in 2015.[9,16,25]

CONCLUSIONS

The frequency of IL-23 R rs 7518660 gene polymorphism G allele and AG genotype was shown to be higher in the pulmonary TB group than in healthy control subjects. While the frequency of IL-23R rs 7518660 gene polymorphism allele A was higher in healthy controls and there was a significant relationship between IL-23 R rs 7518660 gene polymorphism and susceptibility to pulmonary TB, where the higher frequency of IL-23 R rs 7518660 gene polymorphism allele A and G results in the higher risk of developing pulmonary TB in both drug-sensitive and drug-resistant TB.

The result of this study has meaningful information about the relationship between IL-23 R rs 7518660 gene polymorphism with susceptibility of pulmonary TB, but it was not significant with disease severity. For researchers, the results of this study could be reference for further research using different research methods or other markers of the IL-23 R gene polymorphism. In future studies, risk factors should be considered in sample selection, thus the other risk factors are more homogeneous. Susceptibility to pulmonary TB is polygenic, so it is necessary to examine a wider gene polymorphism with more sensitive examination methods such as Restriction Fragment Length Polymorphism (RFLP) and DNA sequencing; therefore, it can explain more about the influence of certain genes on pulmonary TB and even its effect on the severity of the disease caused by those genes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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