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Tumor Necrosis Factor-alpha -308G/A Polymorphism Associated with Increased Risk for Pulmonary Tuberculosis in Medan City, Indonesia

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Abstract

BACKGROUND: Tumor Necrosis Factor-alpha (TNF-α) is a pro-inflammatory cytokine that plays a role in immune response against tuberculosis (TB) infection. Polymorphism in TNF-α gene may be associated with susceptibility to pulmonary TB (PTB).

AIM: The purpose of this study was to investigate whether TNF-α-308G/A gene polymorphism is associated with susceptibility to PTB in Medan city, Indonesia.

METHODS: This is a case–control study with 100 PTB patients and 100 healthy control. TNF-α polymorphism genotyping was performed by polymerase chain reaction fragment length polymorphism method.

RESULTS: There were 200 participants enrolled in this study. Most of the participants were male and were in the age range of 20–39 years. Genotyping examination revealed that in the TB group, AG and GG genotype was found in 80 people (80%) and 20 people (20%), respectively. There were no TB patients with AA genotypes. Whereas, in the control group, subjects with AA, AG, and GG genotypes were 2 (2%), 47 (47%), and 51 (51%), respectively. Statistical analysis showed that there was a significant relationship between TNF-α-308 G/A polymorphism and PTB, in which individuals with AG genotypes were 4.3 times more likely to suffer PTB compared to GG genotype ($p < 0.001$, 95% confidence interval (CI) 2.31–8.15). Further analysis showed that A allele increased the risk of TB incidence by 1.94-fold compared to the G allele ($p = 0.002$, 95% CI 1.27–2.98).

CONCLUSIONS: There was a significant relationship between TNFα-308 G/A gene polymorphism and the susceptibility to PTB and A allele increases the risk of pulmonary TB compared to G allele.

Introduction

Pulmonary tuberculosis (PTB) is a global public health problem, presenting a high incidence in Indonesia [1]. It is estimated that about one-third of the world's population is affected by this disease. However, only 5–10% of an infected person develops the disease [2]. Many factors influence the individual to develop this disease. Besides the environmental factors, host genetic factors play roles in susceptibility to tuberculosis (TB). Twin studies have shown that monozygotic twins have a higher concordance for TB compared with dizygotic twins or other siblings [3]. Some genes have been identified in TB susceptibility and one of these is tumor necrosis factor alpha (TNF-α) gene [4], [5].

TNF-α is a pro-inflammatory cytokine that plays an important role in host defense to TB in humans. It was produced mainly by monocytes and macrophages and also by neutrophils, T cells, and natural killer cells [6]. TNF-α plays an essential role in forming granuloma formation by recruitment of immune cells to the site of infection, increasing the ability of macrophage for controlling the intracellular mycobacteria, stimulating the apoptosis of macrophage, and maintaining the granuloma formation [7], [8]. Some studies found that TNF-α blockage in patients treated with anti-TNF-α results in TB disseminated [9].

The TNF-α cytokines are encoded by TNF-α gene which is located within the major histocompatibility complex class III, a highly polymorphic region on chromosome 6p21. There are many single-nucleotide polymorphisms (SNPs) in the promotor of this region [10]. The SNPs were the most common genetic variation of TNF-α gene and were considered in influencing the transcription of TNF-α cytokine. Some SNPs of TNF-α gene have been identified at positions –238, –308, –857 and –863. One of the most important polymorphisms is -308G/A polymorphism. Some studies found that A allele has higher transcriptional activity compared to the G allele [11], [12], [13].

The study of this association is inconclusive because different results were found for the association of TNF-α gene -308 polymorphism and susceptibility to PTB in several ethnic groups and different populations. Some studies reported a significant association [14], [15], [16], but other studies were not significant [17], [18]. Therefore,

further studies are needed to clarify these results. This study aims to investigate whether TNFα-308G/A gene polymorphism is associated with susceptibility to PTB in Medan city, Indonesia.

Methods

Research design

This is a case–control study with 100 subjects for each group. Consecutive sampling was used to look for the case group included the TB case and healthy people as control group. This study has approved by the Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Population and samples

Cases were 100 PTB patients from several TB facilities in Medan. For the case group, the inclusion criteria were new TB cases, age ≥18 years old, have symptoms of PTB, positive sputum smear, and chest X-ray consistent with PTB. The exclusion criteria in the case group were human immunodeficiency virus (HIV) positive, have diabetes mellitus and other severe comorbid diseases, and consuming immunosuppressive drugs. Control group was 100 healthy subjects from the community with no signs and symptoms of TB, normal chest X-ray, and no history of TB before. All subjects were interviewed and informed consent was obtained.

Genotyping

Genotyping was done from DNA samples that have been stored in laboratorium using minus 70°C refrigerator with the same inclusion and exclusion criteria in this study. TNF-α polymorphism genotyping was performed by polymerase chain reaction fragment length polymorphism (PCR-RFLP) method.

The genomic region encompassing the -308G/A polymorphism was amplified using the following primers: Forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and reverse 5'-TCCTCCCTGCTCCGATTCCG-3'. PCR products then were digested with NcoI restriction enzyme. PCR product of -308 G/A TNF-α gene polymorphism before digested was 107 bp. After digested with the NcoI restriction enzyme, the AA genotype had one band (107 bp), AG genotype had three bands (107, 87, and 20 bp), and GG genotype had two bands (87 and 20 bp).

Data analysis

Data were managed and analyzed using IBM statistics ver. 24.0. The genotype frequencies of each SNP were compared by Chi-square test. The strength of the association was evaluated by calculating the odds ratio (OR) and 95% confidence interval (CI).

Results

Based on sex and age demographic characteristics, the number of male subjects was more than female subject and most of the subjects were in productive age in both groups, mainly in 20–39 years old followed by 40-59 years old (Table 1).

Table 1: Demographic characteristics

Characteristics	Case		Control	
	n	%	n	%
Sex				
Male	68	68.0	73	73.0
Female	32	32.0	27	27.0
Age (years)				
<20	12	12.0	2	2.0
20–39	60	60.0	71	71.0
40–59	26	26.0	26	26.0
≥60	2	2.0	1	1.0
Total	100	100	100	100

Table 2 enlists the allele and the genotype frequencies for TNF-α-308 G/A polymorphism. In the case group, the AA genotype was not found and the AG genotype was more than the GG genotype. The comparison of the allele and genotype frequencies showed a significant difference between TB patients and controls with p = 0.002 and <0.001, respectively.

Table 2: Distribution of -308 polymorphism genotype in PTB case and control

Polymorphism	Case n (%)	Control n (%)	p-value
Genotype			
AA	0	2	
AG	80	47	<0.001
GG	20	51	
Total	100	100	
Allele			
A	80	51	
G	120	149	0.002
Total	200	200	

PTB: Pulmonary tuberculosis, Chi-square test.

Two comparative analyses were done to find the OR value for association of -308 G/A polymorphism and susceptibility to PTB (Table 3). The first analysis is a recessive model (AA+AG genotype vs. GG) with OR 4.16; CI 2.22 0–7.79; p < 0.001, means AA+AG genotypes individuals were 4.16 times more likely to suffer PTB compared to GG genotype. A secondary analysis was the homozygote model (AG vs. GG) with OR 4.3; CI 2.31–8.15; p < 0.001, means individuals with

Table 3: Association between -308 G/A TNF-α gene polymorphism and PTB

Polymorphism	Case		Control		p-value	OR	95% CI
	n	%	n	%			
AA and AG	80	80.0	49	49.0	<0.001	4.16	2.22–7.79
GG	20	20.0	51	51.0			
Total	100	100	100	100			
AG	80	80.0	47	47.9	<0.001	4.3	2.31–8.15
GG	20	20.0	51	52.1			
Total	100	100	98	100			

CI: Confidential interval, PTB: Pulmonary tuberculosis, Chi-square test.

AG genotype were 4.3 times more likely to suffer PTB compared to GG genotype.

There is also a significant association between an allele and PTB (Table 4). The A allele increased the risk of TB incidence compared to the G allele (OR 1.94; 95% CI 1.27–2.98, p = 0.002).

Table 4: Association between -308 G/A TNF-α gene polymorphism allele and PTB

Allele	Case		Control		p-value	OR	95% CI
	n	%	n	%			
A	80	40.0	51	25.5	0.002	1.94	1.27–2.98
G	120	60.0	149	74.5			

OR: Odds ratio, PTB: Pulmonary tuberculosis, CI: Confidential interval, Chi-square test.

The comparison of the association of -308 G/A polymorphism TNF-α gene and TB in different population in the world is listed in Table 5. This study found that there is an association between -308 G/A polymorphism TNF-α gene and TB, same as several studies, but in other populations, there is no association between -308 G/A polymorphism TNF-α gene and TB.

Discussion

Single nucleotide of cytokine genes may influence the immune response by altering the level and function of secreted cytokine. Banerjee *et al*. indicated that high-level TNF-α serum was associated with the presence of -308 A allele and GA/AA genotype [26], and high-level production of TNF-α is considered as a risk factor for active pulmonary progression [27]. On the other hand, Cui *et al*. found that GA and AA genotype was associated with decreased serum TNF-α levels [28].

In this study, there was a significant association between the -308G/A polymorphism and susceptibility to PTB. This study was consistent with some studies but in contrast with several other studies that have shown no significant association between this polymorphism and TB. Table 5 shows the results of the association in different populations and ethnic in the world. Meta-analysis study from a diverse world population found that -308 G/A polymorphism was associated with PTB in Africa, but not in Asian and Caucasian [29].

This discrepancy results may be due to ethnic-specific variation, a different definition of cases and control, and interaction between gene host, agent, and environment. Ethnic-specific genetic variation can influence the immune responses to microbial pathogens. Linkage disequilibrium in specific ethnic population can also affect the association studies [21]. Lifestyle factors such as smoking and alcohol consumption habits and food or diet type also influence the susceptibility to TB. Sinaga *et al*. found that smoking and alcohol consumption habits in one specific ethnic were the risk factor for PTB disease in Indonesia [30].

Gene interaction of the host, gene host-environment interaction, or gene host-agent interaction might influence the results. The opposing effect between the interaction of TNF-α gene and other genes should be accountable for the study. The balance between TNF-α and interleukin-10 was considered to be essential for controlling and dissemination of TB. Besides that, some studies found evidence of host genetics and *Mycobacterium* strain interaction for susceptibility to TB [31].

The difference in the cases defined also might be the cause of the different results. In some studies, cases were identified by smear positive and radiology while other studies used culture confirmed of TB cases. Some studies defined extra PTB as a case group. Coinfection such as HIV may influence the genetic susceptibility to TB. Some studies exclude HIV subjects while other studies included it. It is also important to note that different sources of control may influence the study results such as a history of TB exposure of the subjects.

In this study, the diagnosis was confirmed by microscopic and radiology, HIV status was done in the case group but not in the control group and the exposure history to TB cases in both groups was not defined. The interaction between host gene-gene, host agent, and the effect of lifestyle on our association results is also unknown.

Table 5: Association of -308 G/A polymorphism TNF-α gene and TB in different population

Cases	Controls	Population	Reference	Results
100 PTB patients	100 NC	Indonesian	This study	Association with PTB
100 PTB patients	100 NC	Lur, Iranian	Shahsavar, *et al*. [14]	Association with PTB
138 TB patients	419 NC	Columbian	Correa, *et al*. [16]	Association with TB
636 TB patients	608 NC	Tibetan Chinese	Wu, *et al*. [15]	Association with TB
613 TB patients	603 NC	Han Chinese	Wu *et al*. [15]	No association with TB
140 PTB (30 with pleural TB and 20 with miliary TB)	54 tuberculin negative, 81 tuberculin positive	Colombian	Henao *et al*. [17]	No association of TB
145 TB patients	211 NC	Indian	Kumar *et al*. [18]	No association with TB
93 PTB patients	103 NC	Iranian	Anoosheh *et al*. [19]	No association with PTB
151 PTB patients	83 NC	Iranian	Ghamari *et al*. [20]	No association with PTB
124 TB patients	200 NC	Azeri Iranian	Ghorghanlu *et al*. [21]	No association with TB
128 TB patients	80 NC	Turkish	Ates *et al*. [22]	No association with PTB
149 TB patients	147 NC	Thai	Vejbaesya *et al*. [23]	No association with TB
185 TB patients	155 NC	North Indian	Sharma *et al*. [24]	No association with TB
190 PTB, 183 STB	362 NC	South Chinese	Zhou *et al*. [25]	No association with PTB and STB

TB: Tuberculosis; PTB: Pulmonary tuberculosis; NC: Normal control; STB: Spinal tuberculosis.

Conclusions

Our study demonstrated that TNF-α gene -308 G/A polymorphism was associated with susceptibility to PTB and A allele increases the risk of PTB compared to G allele. Our results could give more understanding about TB pathogenesis. Further studies in other ethnic groups, other gene polymorphism and interaction between host gene, host agent, and environment or lifestyle are needed to fully understand and validate this result.

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