Original Article

The Association Between Vitamin D Receptor Gene FokI Polymorphism and Pulmonary Tuberculosis

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Abstract

Backgrounds: There is a wide range of individual responses to mycobacterium infection. However, the reason why certain people suffers from a disease when they are infected with mycobacterium while others remain healthy is still unknown. Genetic susceptibility is thought to be one of the important explanatory factors for individuals with the risk of developing pulmonary tuberculosis (TB). FokI polymorphism was one of the genetic variations within the vitamin D receptor (VDR) gene associated with pulmonary TB. In the present study, we investigated the association between VDR gene FokI polymorphism and pulmonary TB.
Method: This is a case-control study with 65 pulmonary tuberculosis patients as cases and 65 healthy subjects as controls. The PCR-RFLP technique was used to assess the polymorphism of the vitamin D receptor gene FokI. The association of the vitamin D receptor gene FokI polymorphism with pulmonary tuberculosis was statistically analyzed.
Results: The frequency of FokI genotypes determined from pulmonary TB patients were 41,5% for FF, 44,6% for Ff and 13,8% for ff, while in healthy controls, the frequency of FF, Ff and ff were present in a percentage of; 43,1%, 44,6% and 12,3%, respectively.
Conclusion: There was no significant association between the VDR gene FokI polymorphism and pulmonary TB.
Keywords: FokI polymorphism, pulmonary TB, vitamin D receptor gene

INTRODUCTION

Tuberculosis (TB) is the second leading cause of death from infectious diseases worldwide.[1,2] The global number of pulmonary TB cases has been increasing, compared to previous years. Indonesia ranks second in the largest number of pulmonary TB cases in the world, around 10%.[1] Approximately one third of the world's population has been infected with *Mycobacterium tuberculosis* (*M. tuberculosis*), but only 10% of the infected developed into an active pulmonary TB disease. The susceptibility to the disease after the infection of mycobacterium is influenced by the agent, environmental, and genetic factors.[2,3]

In the past few years, more studies addressing the impact of VDR polymorphisms on TB susceptibility have been conducted in different populations. Polymorphisms in the VDR gene may influence VDR activity and subsequent downstream vitamin D-mediated effects.[4] Four single nucleotide polymorphisms (SNP), which are the variants of VDR, including *Apa*I, *Bsm*I, *Fok*I, and *Taq*I, are the primary focus which have a role in the risk of pulmonary TB compared to the other SNPs.[5–7]

*Fok*I polymorphism (rs2228570) is a transition polymorphism C to T (ACG to ATG) located in exon 2. *Fok*I polymorphism, described as the f allele, allows the protein translation to start from the first ATG, while the F allele starts the protein translation from the location of the second ATG.[5–8] Long vitamin D receptors, coded from the f allele forms, have more three amino acids, and 1.7 times less efficient than VDR coded by the F allele forms. Therefore, this variant may affect the individuals' susceptibility to pulmonary TB.[9–11]

Based on the above description, the researchers conducted a study to investigate the association betweeen VDR gene *Fok*I polymorphism with pulmonary TB.

METHOD

This was a case control study conducted with approval from the Research Ethics Committee of the Faculty of Medicine, University of Sumatera Utara (No: 351/TGL/KEPK FK USU-RSUP HAM/2016), that compared risk factors with disease incidence by comparing a group of people with pulmonary tuberculosis (cases) to a group of people without the disease (control). The sample data was collected at Haji Adam Malik General Hospital Medan and several other health centres in Medan, conducted from April to June 2016.

The sample size in this study was 65 people in the case group and 65 people in the control group. The inclusion criteria of case samples in this study were: new untreated pulmonary TB patients or those who had received antituberculosis treatment <7 days, with positive smear bacteria in sputum based on direct smear examination or positive GeneXpert examination results, male and female patients with pulmonary TB aged 18-65 years and agreeing to participate in the study declared in writing (informed consent). Exclusion criteria in this study, on the other hand, included having HIV, Diabetes Mellitus (DM), and other severe conditions, such as a history of renal disease or liver disease.

All subjects were given informed consent, interviewed, and peripheral blood samples were obtained. DNA isolation (Promega, USA) was performed on the blood and then it was stored at -20°C. The genotype analysis of *Fok*I polymorphism of the VDR gene was performed using Polymerase Chain Reaction Restriction Fragment Length Polymorphisms (PCR-RFLP) technique with endonuclease *Fok*I restriction enzyme (Thermo Scientific). The

primary *Fok*I sequence in this study is *Forward*: 5'-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3', while the primary *Reverse*: 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'. Products digested by the restriction enzymes were then visualized in a Gel Documentation System tool.

The resulting genotype depends on the patterns of digestion. The depiction of electrophoresis, if truncated, would reveal the DNA bands at 169 bp and 96 bp. Otherwise, the DNA bands would be at 265 bp. The existence of restriction area is marked with the lowercase "f", while the absence of restriction area is marked with the letter "F". Whereas FF homozygote produces 265 bp band and ff homozygote produces 169 bp and 96 bp band, Ff heterozygote occurs if there are three bands of 265 bp, 169 bp, and 96 bp.

The statistical analysis used in this research was the logistic regression test. The Hardy Weinberg Equilibrium was tested on the case group and the control group using the HWE Testing Calculator web tool, which was available online.

RESULTS

The characteristics of the case group and the control group can be seen in Table 1. Distribution of genotypes and alleles of *Fok*I polymorphism of the VDR gene can be seen in Table 2. The Ff genotype was the highest genotype in both groups, with 29 people (44.6%) in each the case group and the control group.

Table 1. Characteristic from pulmonary TB patients and healthy people

Characteristic	Case (pulmonary TB)	Control (healthy people)
Age		
18-25 years	14 (21.5%)	30 (46.2%)
26-35 years	28 (43.1%)	23 (35.4%)
36-45 years	8 (12.3%)	10 (15.4%)
46-55 years	13 (20.0%)	1 (1.5%)
55-65 years	2 (3.1%)	1 (1.5%)
Gender		
Men	47 (72.3%)	42 (64.6%)
Women	18 (27.7%)	23 (35.4%)
Total	65 (100.0%)	65 (100.0%)

Table 2. The frequency of allele and genotype VDR gene *Fok*I polymorphism

FokI Polymorphism	Case (pulmonary TB)	Control (healthy people)	P	OR (95% CI)	HWE Case X^2 (p)	HWE Control X^2 (p)
Genotype						
FF	27 (41.5%)	28 (43.1%)	-	1	0,07 (>0.05)	0,01 (<0.05)
Ff	29 (44.6%)	29 (44.6%)	0.923	1,037 (0.496-2.169)		
ff	9 (13.8%)	8 (12.3%)	0.781	1,167 (0.393-3.467)		
Allele						
F	83 (63.8%)	85 (65.4%)	0.795	1.070 (0.643-1.779)	-	-
f	47 (36.2%)	45 (34.6%)				

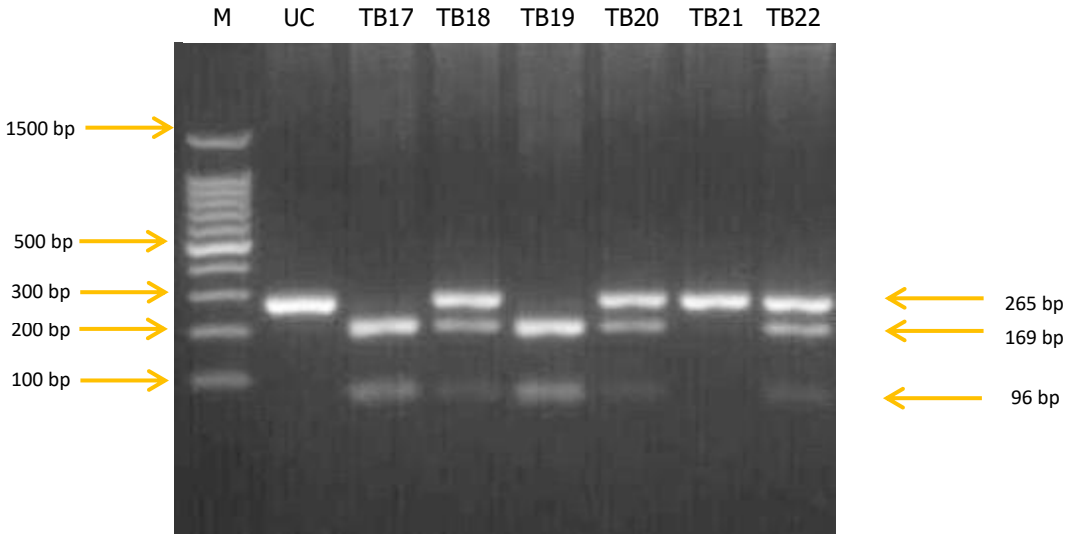

Figure 1. Image of RFLP *FokI* polymorphism with various genotype in samples from pulmonary TB group samples. M = marker, UC = uncut. FF genotype in sample TB21 has 1 band (265 bp), ff genotype in samples TB17 and TB19 have 2 bands (169 bp and 96 bp), Ff genotype in samples TB18, TB20 and TB22 have 3 bands (265 bp, 169 bp, and 96 bp).

There was no statistically significant difference in the genotype distribution of *FokI* polymorphism of the VDR gene between the case group and the control group (P>0.05). The frequency of the F alleles was greater than the frequency of the f alleles in both case group and control group.

The F alleles found in the case group were 83 (63.8%), whereas the f alleles found in the case group were 47 (36.2%). There was no statistically significant difference in the frequency of the F alleles and the f alleles in the *FokI* polymorphism of the VDR gene between the case group and the control group (P>0.05). The genotype of *FokI* polymorphism in the case group was in the Hardy-Weinberg equilibrium (P>0.05), whereas the control group was not in a state of Hardy-Weinberg equilibrium (P<0.05).[12]

The PCR product was truncated by the endonuclease *FokI* restriction enzyme in the case group can be seen in Figure 1.

DISCUSSION

Individual reactions to mycobacterium infection vary greatly; nonetheless, the reason why some people become unwell when infected with mycobacterium while others remain healthy is unknown. Genetic susceptibility is thought to be one of the important explanatory factors for individuals with the risk of developing pulmonary TB.[13]

The VDR gene comprises of a complex of intron/exon structures, located in the long arm of chromosome 12q12-q14 and consists of 16 exons.[5] The VDR gene is known to be responsible for the genetic polymorphism affecting receptor activity and vitamin D-mediated effects. [13]

There are variations in the VDR gene polymorphisms which play a role in the susceptibility of pulmonary TB, one of which is the *Fok*I polymorphism of the VDR gene studied in this study. *Fok*I is one of the VDR variants located in exon 2.[14]

Polymorphism is a variation of DNA sequences which causes genetic diversity in the gene pool of a population. Polymorphism is formed through a mutation process which can occur due to substitution, deletion, or insertion in the order of polynucleotides. Polymorphism has a neutral effect on biological function. However, in some conditions, it may cause an impaired biological function. This happens because there is a change in the DNA sequences that encode proteins. Polymorphism can also be found in DNA areas which do not encode protein.[13]

In this study, there was no statistically significant difference in the genotype distribution of *Fok*I polymorphism of the VDR gene between the case group and the control group. This result was similar to research conducted on Peru's population.[9] Other studies in Cambodia, West Africa, and Korea suggested that there was no relationship between VDR gene polymorphism and pulmonary TB.[9,15,16]

Similarly, in the population of Kazakhs, the *Fok*I and *Apa*I polymorphisms did not have a significant relationship with the risk of pulmonary TB.[7] Studies conducted in Indonesia's population, especially Batak ethnic, showed that there was no relationship between the *Fok*I polymorphism of the VDR gene and the susceptibility of pulmonary TB.[3]

This was not in line with a study conducted on native Paraguayans which showed a link between the *Fok*I polymorphism of the VDR gene and pulmonary TB. The meta-analysis study shows that polymorphisms may have different roles in different populations. For example, there was a significant relationship between pulmonary TB and the *Fok*I polymorphism of the VDR gene in Asian population, but it was not found in African or South American population.[4,16,17]

The significant relationship between the *Fok*I and *Taq*I genotypes of the VDR gene and the susceptibility to pulmonary TB was found in Gambia and London populations.[2]

Based on a population meta-analysis study in China, the variants of homozygote VDR genes to the *Fok*I polymorphism may be more susceptible to pulmonary TB, compared to the heterozygote and wild type variants.[9]

The existence of different results from previous studies may be related to different genetic backgrounds in different populations. There are other four SNPs as variants of VDR, namely *Apa*I, *Bsm*I, *Fok*I, and *Taq*I, which play a role in the risk of pulmonary TB.[5–7]

This study only investigated the *Fok*I polymorphism of the VDR gene, while there are other SNPs variants of VDR which play a role in the risk of pulmonary TB.

CONCLUSION

There was no significant association between VDR gene *Fok*I polymorphism and pulmonary TB.

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