

Polymorphism of Vitamin D Fok1 Receptor Gene in Patients with Pulmonary Tuberculosis of Batak Ethnic

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Abstract: Tuberculosis (TB) is one of the infectious diseases that is still a health problem of the world, especially in developing countries. The polymorphism of the vitamin D receptor gene (VDR) by some studies may affect the workings of vitamin D. The polymorphism of this receptor gene causes a person to become more susceptible to *M. tuberculosis* infection. This study aimed to determine polymorphism of Vitamin D Fok1 Receptor Gene in Patients with Pulmonary Tuberculosis of Batak Ethnic. A total of 42 patients who met the inclusion and exclusion criteria were examined for vitamin D Fok1 receptor gene with PCR-RFLP and analyzed by electrophoresis and the level of vitamin D was examined by ELISA. The results showed that 26 patients had Ff genotype (61,9%), 14 patients have FF genotype (33,3%), and two patients had ff genotype (4,8%). The most common level of Vitamin D in FF genotype was optimal (64,3%), in Ff genotype was optimal (53,8%), and ff genotype had the same percentatage of optimal and insufficiency level. Conclusion: Based on this research, the most common polymorphism of vitamin D Fok1 receptor gene in Batak ethnic is Ff genotype.

1 INTRODUCTION

TB disease is caused by infection with the bacteria *Mycobacterium tuberculosis* (*M. tuberculosis*), which was first discovered by Robert Koch in 1903 so that the first disease called Koch Pulmonum (Chocano-Bedoya & Ronnenberg, 2009). Judging from the year of the discovery of that germs, it can be said to be very ironic, because up to now this disease cannot be eradicated from all over the world, even new problems arise such as the emergence of cases of drug-resistant TBC (Multi Drug Resistance) and TBC cases that accompanied HIV (Human Immunodeficiency Virus).

Several studies have suggested that there is a relationship between vitamin D levels and resistance to TBC. Vitamin D can increase the synthesis of the innate immune system components through the Vitamin D receptor (VDR) complex with the active form of vitamin D (1.25D3), one of which is cathelicidin which has an important role in fighting infection from *Mycobacterium* (Sutaria et al., 2014). Vitamin D can work when binding to VDR.

The polymorphism of this VDR gene causes a person to become more susceptible to *M. tuberculosis* infection. Some of the vitamin D

vitamin receptor polymorphisms that have been identified are TaqI, ApaI, BsmI, and FokI.

The polymorphism of the Fok1 VDR gene is formed by the transition of C to T (ACG-ATG) at the first and second translational initiation sites in exon 2. If the translation starts from the first ATG (individual T allele, written f), the VDR protein synthesis has a maximum length (427 amino acids). Conversely, if translation begins at the second ATG site (individuals with C allele, written as F), then the VDR protein deficits three amino acids at the terminal. The shortening of these three amino acids leads to shorter VDR proteins to be more functionally active (Chocano-Bedoya & Ronnenberg, 2009).

Some studies of these polymorphisms have shown quite varied results, whereas ethnicity also affects the types of polymorphisms associated with TB infection. Studies in Turkey showed that only BsmI variations that have an effect on susceptibility to TB, this result are different from studies in West African populations who reported on variations of ApaI that are significantly associated with TB, whereas in Asian populations, the ff genotype of Fok1 is related to TB, even in a South American study it was found that none of these polymorphism

types were significantly associated with TB (Khalilullah et al., 2014). This suggests that further study of vitamin D polymorphisms in certain ethnic groups is warranted.

2 METHOD

This research is analytic research with cross-sectional design. After getting ethical clearance, the Subjects were collected at Helvetia, Amplas, Teladan and Johor Health Center in Medan in January 2016. The inclusion criteria of subjects were patients (male or female) with Pulmonary TB (category 1) aged 18 to 65 years old, with ethnic Batak obtained from 2 previous generations (grandparents, father-mother). Patients with immune deficiencies such as HIV (examined with HIV rapid test), Diabetes Mellitus (examined with a glucotest device), history of organ transplants, impaired renal function, impaired liver function, malignancy, treatment with steroids, pregnancy and lactation, extrapulmonary TB patients, taking vitamin D, and Body Mass Index ≤ 18.5 were excluded. After signed informed consent, blood samples were taken as much as 3 cc for examined of FokI gene polymorphism and vitamin D levels. The blood samples were centrifuged directly then taken to an integrated laboratory of Faculty of Medicine, University of North Sumatera.

The polymorphism examined using PCR-RFLP and analyzed with electrophoresis gel. On each gel is given marker, positive control, and negative control. The result of the tape was visualized on a UV illuminator device. The resulting genotype depends on the pattern of digestion. Homozygous FF for the absence of a digested FokI side with a band of 265 bp; homozygous ff for a perfectly digested FokI into 196 bp and 69 bp and heterozygous Ff bands if there are three bands (265 bp, 196 bp, and 69 bp).

The Levels of vitamin D (25OH) were examined by ELISA kit (DIAsource®). The absorbance was read at a wavelength of 450 nm.

3 RESULT

Subjects were 42 people; 27 men (62,5% and 32,5% respectively). Subjects with the youngest age in this study was 19 years old and the oldest 63 years old.

3.1. The Levels of Vitamin D

The vitamin D status is classified according to 3 levels; sufficiency (optimal) (30-100 ng/ml), insufficiency (10-29 ng/ml) and deficiency (<10 ng/ml) (suggested reference values for adults from ELISA kit brochure). There were 24 subjects (57,1%) with optimal level, 15 subjects (35,7%) with insufficiency and three subjects (7,1%) with deficiency level.

3.2. The Polymorphism of VDR gen FokI

The results showed that 26 patients have Ff genotype (61,9%), 14 patients have FF genotype (33,3%) and two patients have ff genotype (4,8%).

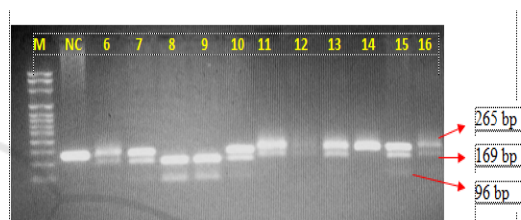


Figure 1: Analyzed bands with electrophoresis gel.

3.3. The Polymorphism and Levels of Vitamin D

The most common level of Vitamin D in FF genotype was optimal (64,3%), in Ff genotype was optimal (53,8%), and ff genotype had the same percentage of optimal and insufficiency level.

Table 1: The polymorphism and levels of vitamin D

Polymorphism	Optimal (n)	Insufficiency (n)	Deficiency (n)
FF	9	4	1
Ff	14	10	2
ff	1	1	0

4 DISCUSSION

The majority of epidemiologic studies found vitamin D status less susceptible to tuberculosis but different from this study where the majority of TB patients had optimal vitamin D status. These different results are likely to be influenced by many factors, one of which is the ethnic factor which from several previous studies can show different results in different ethnic populations (Rashedi et al 2015, Salahuddin et al, 2013; Siswanto et al, 2009; Sutaria et al 2014)

In the distribution of FokI VDR gene polymorphism, the most common genotype was heterozygous (Ff) whereas homozygous ff subjects were found in 2 patients. The same is true of research conducted by Sinaga et al. in Indonesia on Batak ethnic (Sinaga et al., 2014) and Sharma et al. in India in Chhattisgarh ethnic (Sharma et al., 2011). However, these results are different from those of other studies conducted in West Africa (Bornman et al., 2004), India (Selvaraj et al., 2003), South Africa (Babb et al., 2007), England (Martineu et al., 2011) and Iran (Rashedi et al., 2015), which shows the most commonly encountered genotype is the FF type. A meta-analysis by Gao et al. (2010) which states that the ff genotype has risk susceptibility to TB that is clearly different from the results obtained in this study because the genotype ff only found in 2 subjects. Ethnic factors are likely to affect the distribution of polymorphism genotypes in each population.

Table 2: Comparison of FokI genotype in TB Patients from Various Population Studies.

Researcher (year)	Country (ethnic)	FF(%)	Ff(%)	ff(%)
Selvaraj (2003)	India	65	30	5
Bornman (2004)	West Africa	62	33,2	4,8
Babb (2007)	South Africa	58	37	6
Martineu (2011)	England	47	40	13
Sharma (2011)	India/ (Chattisgarh)	44	48	0,07
Sinaga (2014)	Indonesia (Batak)	35,5	55,3	9,2
Rashedi (2015)	Iran	52,4	39,3	8,3

In terms of theory, subjects with an allele F should be stronger than the f alleles, so subjects with ff genotype would be more susceptible to tuberculosis, but this is not the case in this study because the genotype ff is very few and the genotype that has the F allele is susceptible also to tuberculosis. The possibility of Batak tribe is a few who have ff genotype and no previous studies are sufficient to compare the frequency of this genotype. In addition to this study, there is only one more study that also studied polymorphism of VDR FokI gene on Batak tribe that is research by Sinaga et al. (2014) which also shows a proportion distribution similar to this study.

5 CONCLUSIONS

Most subjects showed the optimal status of vitamin D levels. The polymorphisms of FokI VDR gene in Lung TB patients of Batak ethnic encountered in this study are the heterozygous Ff genotype and the least of which is the ff genotype. Ethnic factors may affect a person's susceptibility and correlation to tuberculosis, so it is necessary to conduct similar research on different ethnicities with a large number of samples from this study.

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