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Research Article

FOKI POLYMORPHISM OF VITAMIN D RECEPTOR GENE IN PULMONARY TUBERCULOSIS PATIENTS IN BALI, INDONESIA

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ABSTRACT

Objectives: Nowadays, tuberculosis still becomes a major health problem in Asian country including Indonesia. The response of tuberculosis treatment is affected by many factors including Vitamin D receptor (VDR) gene polymorphism. Several polymorphism on VDR gene was reported in several studies responsible for treatment response or susceptibility to tuberculosis infection, one of which was FokI polymorphism. The distribution of FokI VDR genotype is highly affected by race and ethnicity. So far, there has been no study about this in Bali. Therefore, it is very important to conduct this study to identify the proportion of FokI VDR polymorphism in tuberculosis patients in Bali, Indonesia.

Methods: This study was a cross-sectional study. As many as, 35 subjects were selected from adult pulmonary tuberculosis patients who came to the pulmonary outpatient clinic of Sanglah Hospital, Bali. Analysis of FokI VDR polymorphism was using polymerase chain reaction/restriction fragment length polymorphism technique using restriction enzyme FokI.

Results: The proportion of FF, Ff, and ff genotype in this study was 20%, 54.3%, and 25.7%, respectively.

Conclusions: The dominant genotype of VDR in pulmonary tuberculosis patients in Bali was Ff.

Keywords: FokI polymorphism, Vitamin D receptor, Susceptibility, Treatment, Tuberculosis.

INTRODUCTION

According to the World Health Organization report, until 2013, there were 9 million new cases of tuberculosis and about 1.5 million died due to tuberculosis. In Indonesia, tuberculosis cases reached 5.8%, the third-highest cases in world following India and China [1-4].

Many factors might affect susceptibility to tuberculosis as well as tuberculosis therapeutic outcome, one of which was Vitamin D [5,6]. The role of Vitamin D on immunity and therapeutic response of tuberculosis was mediated by Vitamin D receptor (VDR). This can be affected by several factors, including polymorphism on VDR gene such as FokI polymorphism. Ethnicity and race were two factors most related to genetic variation [5,6]. Different countries with different ethnic or race might have different patterns of genetic variation, and the association between genetic variation and susceptibility or therapeutic response might different as well. Therefore, it was important to conduct research regarding distribution of FokI polymorphism of VDR gene on tuberculosis patients in Bali.

METHODS

Subjects for this study were including tuberculosis patients who attended Pulmonary Outpatient Clinic of Sanglah Hospital, Bali between June 2014 and December 2014. Subjects were selected using consecutive purposive sampling technique with minimum sample size 32. The sample size was calculated based on the proportion of FokI polymorphism in Batak Indonesia on the previous study conducted by Sinaga *et al.* 0.092. The study design was cross-sectional study. This study was approved by Ethical Committee of Sanglah Hospital/Faculty of Medicine, Udayana University, Bali, Indonesia.

DNA was isolated using guanidine isothiocyanate methods. The sequence for forward primer of VDR was 5'-AGC TGG CCC TGG CAC TGA CTC TGG CTCT-3' and for reverse primer of VDR was 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'. DNA chains were denatured at 95°C for 5 min, followed by 35 cycles of reaction (95°C denaturation for 1 min, 65°C annealing for 1 min, and 72°C elongation for 1 min), ended by a final extension at 72°C for 7 min. Polymerase chain reaction (PCR) products were digested using FokI restriction enzyme (New England Biolabs). Incubation was done at 37°C for 1 h. Electrophoresis of PCR-restriction fragment length polymorphism product using 2% agarose gel. FF (wild type), Ff (heterozygote mutant), and ff (homozygote mutant) genotype of VDR showed one band (265 bp), three bands (265, 196, 69 bp), and two bands (196, 69 bp) on electrophoresis result, respectively.

RESULTS

Thirty-five tuberculosis patients were participated in our study. The baseline characteristics of subjects are shown in Table 1. FF (wild type), Ff (heterozygote mutant), and ff (homozygote mutant) genotype of VDR showed one band (265 bp), three bands (265, 196, 69 bp), and two bands (196, 69 bp) on electrophoresis result, respectively (Fig. 1).

Based on electrophoresis results, we found that the frequency and proportion of FF (wild type), Ff (heterozygote mutant), and ff (homozygote mutant) genotype of VDR were 7 (20%), 19 (54.3%), and 9 (25.7%). The frequency and proportion of allele F and f of VDR gene were 33 (47.1%) and 37 (52.9%). VDR FokI genotype patterns in tuberculosis patients based on age and sex are shown in Table 2.

DISCUSSION

Many factors might affect susceptibility to tuberculosis as well as tuberculosis therapeutic outcomes. Several factors contributing to the response of tuberculosis treatment such as age, genetic variation (related to drug-metabolizing enzymes), body mass index, severity of disease, previous treatment, comorbid disease (diabetes mellitus, cancer, and HIV/AIDS), albumin level, duration of tuberculosis treatment, concomitant treatment (immunosuppressive agents), Vitamin D, cigarette, and alcohol [2,7-11].

Ethnicity and race were two factors most related to genetic variation. Different countries with different ethnic or race might have different patterns of genetic variations. Until now, there were four types of polymorphism which have been identified on VDR gene, including FokI, BsmI, TaqI, and ApaI polymorphism. Distribution of FokI alleles of VDR was vary in some populations. In our study, the proportion of allele F and f of VDR gene was 33 (47.1%) and 37 (52.9%), similar to the result of study conducted in Asian. Compare to other study; our study demonstrated higher proportion ofallele f of FokI VDR gene than those in Africa and Caucasia. The proportion of allele f of FokI VDR on African and Caucasian population was 24% and 34%, respectively [7,12]. Contrary to our result, the dominant allele in India was allele F. The proportion of allele F and f in India was 68.5% and 31.5% [13].

In our study, the proportion of FF (wild type), Ff (heterozygote mutant), and ff (homozygote mutant) genotype of VDR was 20%, 54.3%, and 25.7%, respectively. The majority of tuberculosis patients in Bali showed Ff genotype (heterozygote mutant) of VDR. Our result was similar to studies conducted in Chinese, Korean, and Indian population. Distribution of FF; Ff; and ff genotype was in China (34%; 45%; and 21%), Korea (29%; 56%; and 14,6%), and India (44–46%; 42–49%; and 7–12%). Conversely, our study revealed different results with other studies conducted in Iran, Peru, and Indonesia (Batak ethnic). Distribution of FF; Ff; and ff genotype was in Iran (52–57%; 39%; and 3–8%), Peru (9%; 32%; and 59%), and Indonesia Batak ethnic (36%; 55%; and 9%) [6,12-17].

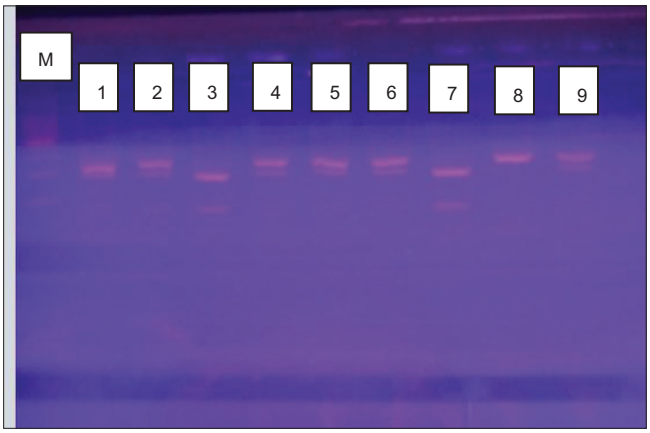

Fig. 1: Electrophoresis visualization of FokI Vitamin D receptor polymorphism. Sample no. 8 showed FF genotype (1 band, e.g., 265 bp). Sample no. 1, 2, 4, 5, 6, and 9 showed Ff genotype (3 bands, e.g., 265, 196, and 69 bp). Sample no. 3 and 7 showed ff genotype (2 bands, e.g., 196, and 69 bp). M: Marker

Table 1: Subject characteristics

No	Subject characteristics	n (%)
1	Sex	
	Male	20 (57.1)
	Female	15 (42.9)
2	Age	
	<30 years old	16 (45.7)
	≥30 years old	19 (54.3)

The association between VDR polymorphism and susceptibility to tuberculosis infection has been reported in many studies, but the result still inconsistent. A significant correlation between FokI polymorphism of VDR and tuberculosis risk was shown in study conducted by Wu *et al*. and Chen *et al.* [14,18]. Some meta-analysis studies also demonstrated similar results, such as study conducted by Sun and Cai, Huang *et al*., Sutaria *et al*., and Xu *et al.* [2,7,19,20].

In meta-analysis conducted by Sutaria *et al.*, it was concluded that the role of VDR polymorphisms and tuberculosis risk varied in different populations. In Asian, genotype Bb and bb BsmI, ff FokI and Tt TaqI have proven to be significantly related to the increase of tuberculosis susceptibility, whereas ApaI genotype showed insignificant correlation. In European, only Bb and bb genotype of BsmI showed significant correlation to tuberculosis risk, whereas other polymorphisms did not show significant results. In African, all of the polymorphisms showed insignificant correlation with susceptibility to tuberculosis [2].

Besides the association with tuberculosis susceptibility, VDR polymorphisms have been known to related with tuberculosis therapeutic response. Otherwise studies about this still limited and the available studies showed inconsistent results. Study conducted in Peru concluded that VDR polymorphism was significantly related to time to culture conversion and time to conversion (on auramine staining) [6]. The conversion (on culture and auramine staining) was faster on FF genotype of FokI VDR than other genotypes of FokI VDR. This result was contradictive to study conducted by Junaid *et al*. The study conducted by Junaid *et al*. concluded that VDR polymorphism was not related to the response of tuberculosis treatment, especially in intensive phase that was assessed from time to sputum conversion [5]. Different from other studies, this study demonstrated insignificant correlation between VDR polymorphism and tuberculosis susceptibility [6].

The role of Vitamin D on immunity has been recognized for several years. The immunomodulatory effect of Vitamin D was related to the antimicrobial and anti-inflammatory activity of Vitamin D in many infectious diseases including tuberculosis. In case of tuberculosis, Vitamin D has been proven to increase eradication of *Mycobacterium tuberculosis* mediated by macrophage [2].

The effect of Vitamin D was mediated through VDR [21]. VDR was included in nuclear receptor family. Human VDR gene was located at chromosome 12q13.11. VDR gene was expressed in several types of immune cells, such as dendritic cells, T lymphocyte, and macrophage [4,14]. Genetical variation in VDR gene might cause change in Vitamin D activity.

Vitamin D, after activated into its active form, 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) or calcitriol, subsequently would bind to VDR inside the macrophage. VDR located inside the cell. After binding to its receptor, this complex would interact with Vitamin D response elements and subsequently induced transcription of many target genes. In case of tuberculosis, Vitamin D will induce transcription of antimicrobial peptide cathelicidin (LL-37) for eliminating *M. tuberculosis* inside the phagolysosome [22].

Table 2: Vitamin D receptor FokI genotype pattern in tuberculosis patients based on age and sex

Subjects characteristics	FF genotype n (%)	Ff genotype n (%)	Ff genotype n (%)	Total n (%)
Age				
<30 years old	3 (18.8)	9 (56.2)	4 (25)	16 (100)
≥30 years old	4 (21.1)	10 (52.6)	5 (26.3)	19 (100)
Sex				
Male	5 (25)	10 (50)	5 (25)	20 (100)
Female	2 (13.3)	9 (60)	4 (26.7)	15 (100)

The certain mechanism of theanti-inflammatory effect of Vitamin D has not been established yet. The active VDR also affected the immune cell by inhibiting lymphocyte proliferation and decrease the production of proinflammatory cytokines to prevent excessive response [2]. Vitamin D has also been proven to have a direct effect on inflammatory response *in vitro* by inhibiting MAPK and NF-kB signaling. The inhibition of MAPK was related to the increased expression of MAPK phosphatase-1 which defosforilated the active MAPK in human monocyte following the binding of $1,25(OH)_2D_3$ to VDR. Similarly, the inhibition of NF-κB signaling was related to the increase of IκBα level (inhibitor of NF-κB) which responsible for the decrease of NF-κB translocation into the nucleus [23].

CONCLUSIONS

The dominant genotype pattern of FokI VDR polymorphism for tuberculosis patients in Bali was Ff. The proportion of non FF genotype higher than FF genotype.

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