Clinical research

The role of FokI polymorphism of vitamin D receptor gene and vitamin D level in multidrug-resistant tuberculosis occurrence in Medan city, Indonesia

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Abstract

Introduction: Multidrug-resistant tuberculosis (MDR-TB) has become a new problem in the world. Many factors have been associated with MDR-TB occurrence. Some studies have demonstrated a role of vitamin D level and vitamin D receptor (VDR) gene polymorphism in MDR-TB. The aim of this study is to explore the role of FokI polymorphisms of the VDR gene and vitamin D level in MDR-TB occurrence in Medan city, Indonesia.

Material and methods: This is a case-control study. Cases were 43 MDR-TB patients from the MDR-TB polyclinic Adam Malik Hospital in Medan, Indonesia. The control group comprised 56 new pulmonary TB cases with positive AFB sputum smear. Patients who were HIV positive, known to have diabetes mellitus or other severe disease, taking immunosuppressive drugs and vitamin D were excluded for both groups. Genetic polymorphisms of the VDR gene were analyzed using PCR-RFLP. Vitamin D level was analyzed using the ELISA procedure.

Results: The frequencies of FokI genotypes were FF 39.5%, Ff 53.5%, ff 7.0% for MDR-TB patients and FF 39.3%, Ff 46.4.% and ff 14.3% for controls. There was no significant association between FokI genotype of the VDR gene and MDR-TB. Vitamin D level in MDR-TB was 53.5% sufficient, 39.5% insufficient and 7.0% deficient. Vitamin D level was 41.1% sufficient, 46.4% insufficient and 12.5% deficient in TB non-MDR. There was no significant association between vitamin D level and MDR-TB.

Conclusions: No role of FokI polymorphism of the VDR gene and vitamin D level in MDR-TB occurrence was found.

Key words: multidrug-resistant tuberculosis, FokI polymorphism, vitamin D receptor gene, vitamin D level.

Introduction

In the last several years, the efforts aimed at tuberculosis (TB) control and the eradication strategy have been threatened by the emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis*. These Multidrug-resistant tuberculosis (MDR-TB) cases cause another complication, regarding worse prognosis, longer duration of treatment, risk of spreading the infection to other people, and a higher economic burden [1]. According to a World Health Organization report in 2014, about 5% of all TB cases globally have been predicted to be MDR-TB cases. The country with the highest number of MDR-TB cases in the world is India

with about 30 000 cases, while Indonesia is in the eighth position with approximately 1 800 cases [2].

Many factors contribute to the occurrence of MDR-TB. Inadequate treatment, failure of treatment, and loss to follow-up are the main factors leading to MDR-TB cases. Other factors include immunity and genetic factors of the host, which in the last several years have been the focus of interest regarding to the occurrence of MDR-TB. Vitamin D level and vitamin D receptor polymorphism have been linked to the occurrence of MDR TB. During tuberculosis infection, macrophage was activated by vitamin D to restrict intracellular growth of *Mycobacterium tuberculosis*. This effect is achieved through binding to the vitamin D receptor (VDR) in macrophages and cathelicidin synthesis was activated to eliminate *M. tuberculosis* in phagolysosomes. Polymorphisms in the VDR gene can affect these processes [3, 4].

Four types of single nucleotide polymorphism (SNP) of the VDR gene, *Apa*I, *Bsm*I, *Fok*I, and *Taq*I, have been linked to the occurrence of pulmonary tuberculosis. *Fok*I polymorphisms is a transition C to T (ACG-ATG) at the first of the two potential translation initiation sites in exon 2. If translation starts at the first ATG site (T allele, designated f), VDR protein is synthesized full-length with 427 amino acids. But if the translation starts at the second ATG site (C allele, designated F), the VDR protein lacks the three NH2-terminal amino acids [5]. Transcription of the F allele is 1.7 times more than the f allele [6], and interaction with transcription factor IIB was more efficient in the F allele, with more potent VDR protein transcription [7].

The number of studies that link VDR genetic polymorphisms with MDR-TB is still limited. One study in Makassar, Indonesia revealed that there was a role of *Apa*I and *Fok*I genotype of the VDR gene with MDR-TB [8]. Another study conducted by Rathored *et al*. stated that there was an association of Ff polymorphism with susceptibility to MDR-TB [9]. A study about the VDR genetic polymorphisms and MDR-TB in Medan, Indonesia is not yet available, but a similar study about these genetic polymorphisms and their role in susceptibility to non-MDR-TB in the Batak ethnic group has been conducted in Medan, Indonesia, showing that there was no role of *Fok*I polymorphism in non-MDR pulmonary TB [10]. The role of vitamin D in non MDR-TB has been widely published. A metaanalysis by Nnoaham and Clarke [11] and Huang *et al*. [12] showed that there was an association of vitamin D deficiency with the occurrence of pulmonary TB. However, the role of vitamin D in MDR-TB is not widely studied yet. Rathored *et al*. concluded that vitamin D deficiency might be one of the predisposing factors to the occurrence of MDR-TB [13]. Iftikhar *et al*. found the same result that the vitamin D level was lower in MDR-TB patients than non-MDR TB patients and healthy controls [14]. In contrast, Herlina *et al*. found no difference in vitamin D level between MDR-TB patients and healthy household contacts in Indonesia [15].

The aim of this study is to identify the role of vitamin D receptor *Fok*I polymorphism and the blood level of vitamin D in MDR-TB occurrence in Medan, Indonesia.

Material and methods

This is a case control study with *Fok*I polymorphisms of the vitamin D receptor gene and vitamin D level as independent variables and multidrug-resistant tuberculosis as a dependent variable. Sample size was determined by using a case and control formula. Sample size was 43 subjects for the case group and 56 subjects for the control group. Cases and controls were found by consecutive sampling. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Cases

Cases were pulmonary MDR-TB patients recruited from the MDR-TB polyclinic in Adam Malik Hospital in Medan, Indonesia from April 2016 to October 2016. Adam Malik Hospital is a referral hospital for MDR-TB from several districts. Patients were diagnosed with sputum GeneXpert, and the age range was 18–65 years. Patients who were HIV positive, known to have diabetes mellitus or other severe disease, or taking immunosuppressive drugs and vitamin D were excluded.

Controls

The control group comprised new pulmonary TB cases from several TB services in Medan city, Indonesia, age 18–65 years, having symptoms of pulmonary TB, with positive sputum smear and chest radiography consistent with active disease. Patients who were HIV positive, known to have diabetes mellitus or other severe disease, taking immunosuppressive drugs and vitamin D were excluded.

All subjects were interviewed and informed consent was obtained. An anticoagulated peripheral blood specimen was collected and polymorphism of vitamin D receptor (VDR) gene was analyzed using PCR-RFLP. Vitamin D level was analyzed using the ELISA method.

VDR genotyping

The DNA was extracted (Promega, USA) and stored at minus 20°C. *Fok*I polymorphism of the vitamin D receptor gene was identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The primer

Table I. Demographic characteristics based on gender and age

Characteristic	MDR TB *n* (%)	Non-MDR TB *n* (%)	*P*-value
Gender:			
Male	25 (58.1)	41 (73.2)	0.115[a]
Female	18 (41.9)	15 (26.8)	
Age [years]:			
16–34	16 (37.2)	36 (64.3)	
35–54	21 (48.8)	18 (32.1)	0.015[b]
55–74	6 (14.0)	2 (3.6)	
Total	43 (100)	56 (100)	

[a]χ^2 test, [b]Fisher exact test.

sequences used in this study were as follows: *forward primer*: 5′-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3′ and *reverse primer*: 5′-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3′.

Denaturation at 94°C for 5 min, followed by 35 cycles of PCR at 94°C (30 s), annealing at 61°C and final extension at 72°C for 7 min were the PCR conditions. The amplified PCR products was digested with *Fok*I (Thermo Scientific) restriction enzyme at 37°C for 1 h. Digested products were analyzed using electrophoresis in 2% agarose gel and ethidium bromide stained. The bands were visualized by a gel documentation system.

Depending on the digestion pattern of *Fok*I polymorphism, individuals were scored as ff when homozygous for presence of the *Fok*I site (169 bp and 96 bp), FF when homozygous for absence of the *Fok*I site (265 bp), or Ff in the case of heterozygosity (265 bp, 169 bp and 96 bp).

The examination of vitamin D level was performed with an enzyme-linked immunosorbent assay (ELISA) (DIAsource ImmunoAssays S.A., Belgium) Vitamin D serum level was defined as deficient if below 20 ng/ml, insufficient if in the range 20–30 ng/ml, and sufficient if above 30 ng/ml[16].

Statistical analysis

The χ^2 test was used to compare the genotype frequencies of each SNP. The strength of the association between VDR *Fok*I polymorphisms, vitamin D level and MDR-TB risk was evaluated by calculating the odds ratio (OR) with 95% confidence interval (95% CI). The 2-sided exact *p*-value < 0.05 was considered statistically significant. Data were managed and analyzed using SPSS.

Table II. Distribution of *Fok*I polymorphism of VDR gene

*Fok*I polymorphism	MDR-TB *n* (%)	Non-MDR-TB *n* (%)	*P*-value
FF	17 (39.5)	22 (39.3)	0.493
Ff	23 (53.5)	26 (46.4)	
ff	3 (7.0)	8 (14.3)	
Total	43 (100)	56 (100)	

χ^2 test.

Results

The number of subjects in this study was 43 cases of MDR-TB, and 56 cases of non-MDR TB. The characteristics of subjects in this study are presented in Table I. Table I shows that males were more common than females in both groups. The most common age group was 35–54 years old in MDR-TB and 16–34 years old in non-MDR-TB, while the least common age group was 55–74 years old in both groups. There was no significant difference in gender between the groups, but there was significant association in age distribution.

Based on analysis of *Fok*I polymorphism, there was no significant difference of *Fok*I polymorphism genotype between groups (Table II).

Table III shows that there was no role of FokI polymorphism of the VDR gene in MDR-TB occurrence compared with non-MDR TB.

Table IV shows that there was no role of vitamin D level in MDR-TB occurrence compared with non-MDR TB.

Discussion

Studies about VDR gene polymorphism and MDR TB are still limited. One study in Indonesia revealed an association of MDR-TB with *Apa*I and *Fok*I polymorphisms [8]. Another study by

Table III. The role of *Fok*I polymorphism of VDR gene in MDR-TB

Polymorphism	Genotype	MDR-TB *n* (%)	Non-MDR-TB *n* (%)	*P*-value	OR	95% CI
*Fok*I	FF	17 (39.5)	22 (39.3)		1	
	Ff	23 (53.5)	26 (46.4)	0.754	1.14	0.49–2.67
	ff	3 (7.0)	8 (14.3)	0.335	0.48	0.11–2.11
Total		43 (100)	56 (100.0)			

Logistic regression test, FF as the reference value.

Table IV. The role of vitamin D in MDR-TB

Vit. D level	MDR-TB *n* (%)	Non-MDR-TB *n* (%)	*P*-value	OR	95% CI
Sufficient	23 (53.5)	23 (41.1)		1	1
Insufficient	17 (39.5)	26 (46.4)	0.322	0.65	0.28–1.51
Deficient	3 (7.0)	7 (12.5)	0.259	0.42	0.09–1.86
Total	43 (100.0)	56 (100.0)			

Logistic regression test, sufficient as the reference value.

Rathored *et al.* showed an association between Ff polymorphism and low level of vitamin D with MDR-TB [9].

This study showed that there was no role of *Fok*I polymorphism of the VDR gene in MDR-TB occurrence. This study also did not find a role of vitamin D level in MDR-TB occurrence.

This difference might be due to the difference in population and subjects. The frequency of *Fok*I VDR gene polymorphism in the world is different between ethnic groups and populations. The F allele of VDR gene polymorphism is relatively low in the African population (24%) compared with Caucasian (34%) and Asian (51%) populations [17].

This difference in results might also be due to the difference in environmental influence such as nutrition intake, smoking and alcohol consumption, which was different from other studies.

Another important reason is that MDR-TB patients in this study were all secondary MDR-TB, patients with prior treatment with anti-tuberculosis drugs. Hence, the occurrence of MDR TB in this study is particularly because of mutation in the microorganism due to inadequate treatment. History of anti-tuberculosis treatment is a strong risk factor to develop MDR-TB. People with prior history of antituberculosis treatment are 10 times more likely to develop MDR-TB compared to those without prior treatment [18].

Nevertheless, this study still has several limitations. We did not recognize any smoking habit or alcohol consumption in both case and control groups, which could also contribute to MDR-TB occurrence and vitamin D level. Another limitation is the fact that we did not perform sputum culture or a sensitivity test in the control group (new pulmonary TB cases). However, recent data suggested that primary resistance of MDR-TB in new TB cases occurred only in about 4% of all patients [19]. This study also has a difference of age between groups, which could also have influenced the results.

This study identified only one type of polymorphism from several kinds of VDR gene polymorphisms reported. It is possible that other VDR gene polymorphisms could also contribute to the MDR-TB occurrence. In addition, gene-gene interaction [20] and gene-agent interaction could also influence the occurrence of tuberculosis [21].

This study was conducted in Indonesia, which straddles the equator and is exposed to sunlight throughout the year. A study on healthy woman in Indonesia at the same location where this study was done found that no subject had a sufficient vitamin D level; most of them were deficient or insufficient [22]. This might explain the result of the study by Herlina in Indonesia, who found no difference in vitamin D level between MDR-TB patients and healthy household contacts [15].

A low level of vitamin D may also be caused by a disturbed cellular pathway of vitamin D synthesis, from vitamin D diffusion in the target cell, vitamin D receptor and formation with RXR (retinoid X receptor), enzymes that are involved in these processes and vitamin D receptor polymorphisms [4].

Further studies are still needed particularly to identify the smoking history, alcohol consumption, nutritional intake, and occupation in both groups, which may influence the level of vitamin D and the susceptibility to MDR-TB. Another study of polymorphism of another gene is also needed to identify the role of other gene polymorphism in MDR-TB. It is particularly important to identify risk factors for MDR-TB, and hence could lead to a better prevention and treatment strategy of MDR-TB.

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Conflict of interest

The authors declare no conflict of interest.

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