ORIGINAL ARTICLE

*Fok*I Vitamin D Receptor Gene Polymorphism in Leprosy Household Contacts

Ramona Dumasari Lubis[1], Irma D. Roesyanto-Mahadi[1], Yahwardiah Siregar[2], Putri Chairani Eyanoer[3]

[1] Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia
[2] Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Indonesia
[3] Department of Community and Preventive Medicine, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

ABSTRACT

Introduction: Leprosy is a chronic infectious neural and skin disease caused by *Mycobaterium leprae* (*M.leprae*). Household contacts of leprosy are at risk for M. leprae infection and subclinical leprosy. Vitamin D is known for its affect to immune system and many studies have showed that polymorphism of vitamin D receptor gene, such as *Fok*I, is contribute to clinical presentation and susceptibility for leprosy. The purpose of this study is to know the distribution of *Fok*I VDR polymorphism in leprosy household contacts. **Methods:** This is observational study conducted in Leprosy Division, Dermatology and Venereology Department, Haji Adam Malik General Hospital and Dr. Pirngadi General Hospital in Medan, also in several primary healthcare centers in North Sumatera. The inclusion criteria for household contacts are living with multibacillary leprosy patient, aged more than 15 years old, have not diagnosed with leprosy, agree to take part in the study. We recruited, interviewed, examined, and got their blood taken from 95 of leprosy household contacts. The blood sample then further examined to detect *Fok*I VDR gene polymorphism. The collected data were tabulated. **Results:** Our study showed that most of leprosy household contacts have genotype Ff (53.7%) with allele F (59.5%) and female (63.2%), within productive age (30.5%) with education level of high school (35.8%), and unemployed (30.5%). **Conclusion:** Household contacts of leprosy patients are at risk of leprosy and can contribute to ongoing transmission. The most of *Fok*I VDR gene polymorphism of leprosy household contacts were genotype Ff with allele F.

Keywords: Leprosy, Vitamin D receptor, *Fok*I, Gene polymorphism, Household contact

Corresponding Author:
Ramona Dumasari Lubis, PhD
Email: ramona_lubis@yahoo.com
Tel: +62812-6004-010

INTRODUCTION

Leprosy is an infectious disease and can cause very complex problems for the lives of leprosy patients and the surrounding community, from the health, social to political fields (1). The negative stigma of leprosy will hinder the treatment program, causing delays in the delivery of health services due to delayed discovery and early diagnosis of new leprosy patients (2). This has led to continued transmission and increased disability rates (3).

Southeast Asia is the largest contributor to new leprosy cases, with an increase in new cases from 2014, amounting to 154.834 cases, to 156.118 cases in 2015. Based on the WHO report (2016), Indonesia still ranks third in the discovery of the number of new cases of leprosy in the world after India and Brazil (4). The prevalence of leprosy in Indonesia in 2015 was 0.79 per 10,000 population and during the previous three years there was no significant change. The discovery of new cases of leprosy were reported as many as 17.202 cases and 1.920 of them were in children (5).

The leprosy prevalence in North Sumatra in 2015 was 0.15 per 10.000 population and 197 new cases were still detected, consisting of cases of Paucibasillary (PB) and Multibacillary (MB) each of 25 cases and 172 cases. New cases of leprosy in children are still found, with a total of 20 cases (10.15%) and a grade II disability rate reported of 3.16 per 1,000,000 population (5). Household contacts can be at risk of becoming infected and have leprosy in subclinical stages (6). Subclinical stage leprosy (SSL) is a term that describes the condition of an individual who clinically appears healthy, but on serological examination, it is found that specific antibodies against *M. leprae* in high levels (7). SSL will develop into leprosy or can heal itself, and is thought to contribute to leprosy transmission (7-9).

Vitamin D can induce antimicrobial activities against *M. leprae* by binding with vitamin D receptor (VDR) (10-12). Polymorphism of VDR can affect stability of

RNA messenger (mRNA) of VDR that influence clinical presentation and susceptibility to leprosy (13,14). There is still limited study that explores VDR gene polymorphism of leprosy household contacts. Through this study, we would like to provide information about *Fok*I VDR gene polymorphism in leprosy household contacts.

MATERIALS AND METHODS

This is a cross-sectional study to describe *Fok*I VDR gene polymorphism in leprosy household contacts. The study was conducted in Leprosy Division, Dermatology and Venereology Department, Haji Adam Malik General Hospital, Dr. Pirngadi General Hospital in Medan, and several primary healthcare centers in North Sumatera. This study included a consecutive sampling of 95 leprosy household contacts. After signing informed consent form, subjects that meet inclusion criteria and do not meet exclusion criteria were interviewed, examined, and have their blood taken for further examination. The inclusion criteria for household contacts are living with multibacillary leprosy patient, aged more than 15 years old, have not diagnosed with leprosy, agree to take part in the study. The exclusion criteria are having other chronic infection, immunosuppressed condition, in pregnant condition or still breastfeed. After physical examination, we took 5 cc of blood samples from subjects and processed it at Laboratorium Terpadu Faculty of Medicine, Universitas Sumatera Utara (USU), Medan for Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) to detect *Fok*I VDR gene polymorphism. PCR for the qualitative test is done. To prove that this FoxI gene are a polymorphism in leprosy household contacts, we performed the calculation of Hardy-Weinberg Equilibrium (HWE). This calculation was done using the HWE calculator with the following link: https://www.researchgate.net (Michael H. Court 2005-2008) which produced the p value of the HWE. All of the demographic data and PCR-RFLP of subjects were tabulated. This study had been approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Sumatera Utara under the ethical clearance number: 454/TGL/KEPK FK USU-RSUP HAM/2018 dated 31st July 2018.

RESULTS

There was a total of 95 household contacts. Characteristics of subjects in each group are presented in Table I. Most of the subjects were aged between 15 to 24 years old (30.5%) and were female (63.2%). Most of them have high school degree (35.8%) and unemployed (30.5%).

Distribution of *Fok*I genotype and allele in our subjects can be seen in Table II. Majority of the *Fok*I genotype was Ff with 51 subjects (53.7%). The allele in our research subjects was F allele in 113 subjects (59.5%) and f allele in 77 subjects (40.5%). The calculation of Hardy-Weinberg Equilibrium was done in this study with p = 0.312.

Table I: Leprosy household contacts characteristics

Characteristics	Household Contacts	
	N	%
Age (years):		
15-24	29	30.5
25-34	20	21.1
35-44	18	18.9
45-54	16	16.8
55-64	9	9.5
> 64	3	3.2
Gender:		
Male	35	36.8
Female	60	63.2
Education:		
Uneducated	6	6.3
Primary school	18	18.9
Middle school	28	29.5
High school	34	35.8
Bachelor degree	9	9.5
Occupation:		
Civil servant	2	2.1
Private employee	5	5.3
Entrepreneur	28	29.5
Housewives	18	18.9
Farmer	10	10.5
Unemployed	29	30.5
Others	3	3.2
Total	95	100.0

Table II: Distribution of FokI genotype and allele based on polymorphism of VDR gene in leprosy household contact

Genotype	Household contacts	
	n	%
FF	31	32.6
Ff	51	53.7
ff	13	13.7
Total	95	100.0
Allele		
F	113	59.5
f	77	40.5
Total	190	100.0

DISCUSSION

Most of household contacts in this study were aged between 15 to 24 years old. Younger or older age can affect immune system, causing higher risk of infection

(15,16). Aging in the immune system includes persistent mild degree of inflammation, decreased ability to overcome infection or cancer, inability to respond effectively to new antigens, increased autoimmune incidence and wound healing (17). The ability of natural immune responses, such as neutrophils and macrophages, decreases with age. This results in increased susceptibility to bacterial and viral infections in old age (16). Most of these household contacts were family members with first-degree blood relationship and have close contact with patients, therefore have a higher risk for leprosy.

In our study, we found more household contact were female. Transmission of leprosy can be influenced by gender. In many studies, male is known to be more prone to leprosy than female (18). Study by Cardona-Castro et al, also showed a similar result with this study (19). An emphasis should be done on this finding because sociocultural factors is one of many factors that contribute to underreporting of leprosy cases in women (20).

We found that majority of subjects were high school graduates. Similar condition also found in a study that was conducted in endemic region in Brazil (21). Our data also showed that there were still high percentage of subjects that unemployed. There are evidences that indicate lower level of education and socioeconomic condition is associated with a greater risk of leprosy (22). There is social stigma that attached with leprosy, which can contribute to poor socioeconomic condition of leprosy patients (21).

Vitamin D and VDR have an important role in regulating immune responses (23). *Fok*I VDR gene polymorphism is in exon 2, caused an alternative start codon with either longer or shorter amino acid sequences of VDR protein. This type of VDR with fewer amino acids has higher capacity as transcription factor and has more efficiency in binding vitamin D (24-26). According to study by Etten et al, *Fok*I VDR gene polymorphism can affect immune cells, especially from FF genotype (26). Recently, it is thought that this polymorphism can affect susceptibility to leprosy and its clinical manifestations (27).

In this study, Hardy Weinberg Equilibrium for the *Fok*I vitamin D receptor gene polymorphisms showed a p-value > 0.05. Based on this result, it was concluded that *Fok*I is indeed a polymorphism of vitamin D receptor gene. Majority of subjects research had Ff genotype and C allele. The results of this study are in line with the examination of VDR gene polymorphisms in healthy people by Bid et al (2005) in the North Indian population, obtained the most genotypes and alleles of each *Fok*I VDR gene were Ff with allele F (28). Similar result was also found in healthy Taiwanese population (29). Study by Bhanushali et al (2009) in the Indian population found that the highest *Fok*I VDR polymorphism genotype was FF (30).

There are several studies that evaluate risk of leprosy in VDR gene polymorphism. Study by Singh et al in India found that *Fok*I associated with leprosy (Singh et al., 2018). Neela et al also found that ff genotype, particularly, associated with higher risk of leprosy (Neela et al., 2015). Unfortunately, we could not evaluate level of risk for leprosy because there are limitations in study design and number of samples.

CONCLUSION

Leprosy is chronic skin and neural disease that still becomes a global problem, especially in Indonesia. Household contacts of leprosy patients are at risk of leprosy and can contribute to ongoing transmission. Many studies have shown the role of vitamin D in immune system and it is thought that polymorphism of VDR gene can also contribute in susceptibility of leprosy and its clinical manifestations. Also, most of *Fok*I VDR gene polymorphism of leprosy household contacts were genotype Ff with allele F. The majority of this research subjects were within productive age, female, have high school degree, and unemployed.

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