Vitamin D Receptor Gene Polymorphisms FokI rs2228570, Apal rs797523, and TaqI rs731236 in Multibacillary Leprosy Patients

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Abstract

AIM: Knowing distribution frequency of genotype and allele Vitamin D receptor (VDR) gene polymorphism FokI rs2228570, Apal rs797523, and TaqI rs731236 in leprosy patients.

METHODS: This is an observational research that was done in Leprosy Division, Department of Dermatology and Venereology, Haji Adam Malik General Hospital, Dr. Pirngadi General Hospital in Medan, and other primary healthcare facilities in North Sumatera. The research subjects underwent an interview process, physical examination and blood collection to detect VDR gene polymorphism FokI rs2228570, Apal rs797523, and TaqI rs731236. The data were then tabulated and analyzed, also calculated using Hardy-Weinberg equilibrium.

RESULTS: This study involved 52 leprosy patients, with most of them aged between 35 and 44 years (38.5%), male (61.5%) more than female (38.5%). The research subjects have VDR gene polymorphisms FokI rs2228570 with genotype FF (42.3%) with F allele (59.6%), Apal rs797523 genotype AA (46.1%) with A allele (65.4%) and TaqI rs731236 genotype TT (86.5%) with T allele (93.3%).

CONCLUSION: Most of the leprosy patients have genotype FF with F allele, genotype AA with A allele and TT with T allele. Further research can be done to assess the relationship between the VDR gene polymorphism and leprosy risk.

Introduction

Leprosy is a chronic infectious neurological and skin disease caused by *Mycobacterium leprae* (*M. leprae*)[1]. This condition affects the peripheral nerves skin, and other tissues such as the reticuloendothelial system, bones, joints, mucous membranes, eyes, testes, muscles, and adrenal glands [2]. Transmission of this disease is through inhalation, direct skin contact, in utero, gastrointestinal tract, and trauma [3]. There is a negative stigma about leprosy that causes delays in early diagnosis and treatment of new leprosy patients, which increases the morbidity and transmission rates ongoing [4], [5]. According to a report by the World Health Organization or WHO in 2016, Indonesia still ranks third, after India and Brazil, contributing to new cases of leprosy in the world. A report in 2017 found that the prevalence of leprosy in Indonesia has been stable in the last 3 years and 10 out of 34 leprosy provinces have not been eliminated [6].

In 2016, WHO launched the Global Leprosy Strategy 2016–2020 program, which targets early detection of leprosy and management to prevent disability and reduce transmission of infection in the community. One component of this program prioritizes early detection through active case finding in endemic areas and management of household contacts for leprosy patients [7]. Household contacts with leprosy have a higher risk of infection and subclinical leprosy [8], [9]; because it can contribute to leprosy transmission [10], [11], [12].

Vitamin D is a lipid-soluble derivative that essential for a number of physiological processes [13]. Vitamin D binds to the Vitamin D receptor (VDR) and induces antimicrobial activity against infectious pathogens, such as *M. leprae* [14]. This induces cathelicidin production (LL-37) and β-defensin2 [15],[16]. Cathelicidin can cause disintegration of pathogenic cell walls, followed by autophagosome [15], [16], [17].

There are several studies investigating the effects of VDR polymorphisms on leprosy. VDR polymorphism can affect the stability of messenger RNA (mRNA) VDR, which can affect clinical presentation and susceptibility to leprosy [18], [19]. In FokI VDR polymorphism, FF genotype is often seen in paucibacillary (PB) leprosy patients; whereas in VDR Apal polymorphism it is found that allele A is significant more common in patients with multibacillary type (MB) leprosy [19]. Then, a study by
Lubis et al. VDR Gene Polymorphisms in Leprosy Patients

Roy et al. in India, Felix et al. in Mexico, and Fitness et al. in Malawi, showed that the VDR TaqI polymorphism with the TT genotype was more often found in tuberculoid type leprosy, while the TT genotype was slightly more common in lepromatous type leprosy [20], [21], [22]. In Indonesia, there were more MB type leprosy patients than the PB and so far, although Indonesia has the third largest number of leprosy patients after India and Brazil, it is not yet known which type is found especially in Medan, North Sumatra which correlates with the polymorphism of VDR, so this study provides information about the polymorphism of the VDR FokI rs2228570 gene, ApaI rs797523, and TaqI rs731236 and its correlation with certain types of leprosy patients.

Methods

A total of 52 leprosy patients were recruited from the Leprosy Division, Dermatology and Venereology Department, Haji Adam Malik General Hospital, Dr. Pirngadi General Hospital in Medan, and several primary healthcare facilities in North Sumatera. Polymorphism analysis of the VDR FokI rs2228570, ApaI rs797523, and TaqI rs731236 gene polymorphisms was carried out on blood samples in Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara, Medan using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method. The results of the Hardy-Weinberg Equilibrium (HWE) calculation state that the population studied is in accordance with the HWE (p > 0.05). All demographic and PCR-RFLP data from leprosy patients were tabulated and analyzed.

Results

This study involved 52 leprosy patients and the characteristics of the study subjects were presented in Table 1. The majority of leprosy patients were between 35 and 44 years old (38.5%) and more was found in men (61.5%) than women (38.5%).

| Characteristics | Leprosy patients n (%) |
|-----------------|------------------------|
| Age             |                        |
| 15–24           | 9 (17.3)               |
| 25–34           | 12 (23.1)              |
| 35–44           | 20 (38.5)              |
| 45–54           | 7 (13.4)               |
| 55–64           | 1 (1.9)                |
| >64             | 3 (5.8)                |
| Gender          |                        |
| Male            | 32 (61.5)              |
| Female          | 20 (38.5)              |
| Total           | 52 (100.0)             |

The frequency distribution of the genotypes of the VDR gene polymorphism FokI rs2228570, ApaI rs797523, and TaqI rs731236 can be seen in Table 2. All study subjects had MB leprosy and on PCR-RFLP it was found that most patients had the FokI rs2228570 genotype FF VDR gene variant (42.3%) with F allele (59.6%), Apal rs797523 AA genotype (46.1%) with allele A (65.4%) and TaqI rs731236 genotype TT (86.5%) with T allele (93.3%), which can be seen in Table 3. After HWE calculations, it can be concluded that the VDR FokI rs2228570, Apal rs797523 and TaqI rs731236 are polymorphisms because they conform to HWE (p > 0.05).

Table 2: Frequency distribution of VDR gene polymorphisms FokI rs2228570, Apal rs797523, and TaqI rs731236 in leprosy patients

| Characteristics | FokI rs2228570 n (%) | ApaI rs797523 n (%) | TaqI rs731236 n (%) |
|-----------------|----------------------|---------------------|---------------------|
| FF              | 20 (38.5)            | 8 (15.4)            | TT 45 (86.5)        |
| FF              | 22 (42.3)            | AA 20 (38.5)        | TT 7 (13.5)         |
| FF              | 10 (19.2)            | AA 24 (46.1)        | TT 0 (0.0)          |
| Total           | 52 (100.0)           | Total 52 (100.0)    | Total 52 (100.0)    |

Table 3: Frequency distribution of VDR gene polymorphism alleles FokI rs2228570, Apal rs797523, and TaqI rs731236 in leprosy patients

| Characteristics | FokI rs2228570 n (%) | ApaI rs797523 n (%) | TaqI rs731236 n (%) |
|-----------------|----------------------|---------------------|---------------------|
| F               | 62 (59.6)            | 36 (34.6)           | 97 (93.3)           |
| F               | 42 (40.4)            | A 68 (65.4)         | T 7 (6.7)           |
| Total           | 104 (100.0)          | Total 104 (100.0)   | Total 104 (100.0)   |

Discussion

In leprosy patients, we found more subjects aged 35 to 44 years. Younger or older age can affect the immune system, leading to a higher risk of infection [23], [24]. Bakker et al. found that most of the leprosy patients recruited for their study were between 15 and 29 years of age [25]. One study showed that there is a bimodal distribution of ages who have a higher risk of leprosy, namely 10 to 19 years and over 30 years of age [26].

Gender is known to be one of the risk factors that contribute to leprosy transmission. Research shows that men are more prone to leprosy than women [27]. Similar results can be found in our study, leprosy patients are more common in men than women. However, the report shows that socio-cultural factors are responsible for the possibility of under-reporting of leprosy among women [28]. Therefore, this issue must be considered in interpreting the research data.

VDR can be found in almost all types of cells, one of which is the immune system. Vitamin D can suppress pro-inflammatory cytokine synthesis, immunoglobulin production, and lymphocyte proliferation [29]. The study subjects found that most of them had genotypes of FF (42.3%), AA (46.1%) and TT (86.5%). VDR gene polymorphisms can affect the strength of the host immunity response to M. leprae, thereby determining a person’s susceptibility to contracting leprosy [8]. The VDR FokI
gene polymorphisms are at exon 2, causing alternative starting codons with longer or shorter VDR protein amino acid sequences. This VDR with a shorter amino acid sequence has a higher capacity as a transcription factor [30], [31], [32]. The polymorphism of the VDR Apal gene is located at intron 8, while TaqI is in exon 9. Until now, it is not certain the function of this polymorphism [33]. Therefore, it is known that the polymorphisms of the VDR gene at the three points FokI, Apal, and TaqI can affect mRNA instability resulting in an imbalance in the production of Th1 and Th2 cytokines which are very important in the incidence of leprosy [19], [34], [35].

Research by Roy et al. and Felix et al. showed that the TT genotype was associated with LL type leprosy [20], [21]. However, another study by Sapkota et al. and Goulart et al. did not find a relationship between TaqI polymorphisms and subtypes of leprosy, however, Goulart et al. It was found that the frequency of the T allele was higher in MB than in PB [8], [36]. Research by Neela et al., in India, found that the frequency of FF is higher in PB type leprosy patients compared to controls. Whereas at the Apal locus, the A allele was significantly higher in MB leprosy patients compared to healthy controls [19]. The results of Singh et al. In India, it was found that single nucleotide polymorphisms from the VDR FokI rs2228570 and TaqI rs731236 genes were associated with leprosy. The highest frequency of genotypes and alleles in leprosy patients for FokI rs2228570 was FF with the F allele, while for TaqI rs731236 the most genotypes and alleles were TT and T allele [35].

Conclusion

Leprosy is a chronic skin and nerve disease that is still a global problem, especially in Indonesia. Household contacts of persons affected by leprosy are at risk of leprosy and it can contribute to ongoing transmission. Many studies have demonstrated the role of vitamin D in the immune system and it is thought that the VDR gene polymorphisms may also contribute to leprosy susceptibility and its clinical manifestations. In addition, it was found that most of the leprosy patients had genotypes and polymorphism alleles of the VDR gene, namely FF with the F allele, AA with alleles A and TT with T alleles. This can be used as basic data for further research to assess the relationship between VDR gene polymorphisms and the risk of leprosy.

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