Proportion of TLR-9 Gene Polymorphisms at rs352139 (G1174A) in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients in West Java, Indonesia

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Abstract

Background: Human immunodeficiency virus (HIV) infection is the main cause of the immunodeficiency syndrome (AIDS). TLR-9 gene encodes a toll-like receptor-9 that plays a key role in innate immunity. This study aimed to describe the proportion of TLR-9 polymorphisms at rs352139 in patients with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) in Bandung, West Java, Indonesia.

Methods: This was a descriptive study involving a total of 96 patients with HIV/AIDS treated in a tertiary hospital in Bandung, West Java, Indonesia in 2013. TLR-9 gene polymorphisms atrs 352139 were examined using a mass screening platform and the genotypes proportion was presented in percentage and compared with other populations.

Results: The average age of the HIV/AIDS patients recruited was 30 years(SD+6.1) and the baseline mean of CD4+ count was 318.02 mm3(Normal was 1,500 mm3) (SD+273.1). The proportion of polymorphisms at rs352139or G1174A presented a wild type genotype GG (42.7%), GA (44.9%), and AA (12.4%), resulting in a total proportion nucleotide change of 57.3%.

Conclusions: A total proportion of nucleotide change or polymorphism is higher than the wild type. A further cohort study is of great interest to associate the rs352139 polymorphisms with a decrease in CD4+cells in HIV/AIDS patients, confirming a rapid disease progression.

Keywords: CD4+, HIV/AIDS, polymorphisms, rapid progression, TLR-9, West Java

Introduction

Human immunodeficiency virus (HIV) infection is the main cause of getting immunodeficiency syndrome (AIDS).[1] Indonesia has 48,000 new HIV infections and 38,000 AIDS-related deaths in 2016, and HIV became thus a problem due to increased cases in the population. Since 2010, new HIV infections is increased by 68%.[2] In West Java the number of people with HIV in 2014 reach 13,507 individuals.[3] It is well known, that individual infected with HIV has a relatively long latent period with an average of 10 years until symptoms appear.[4] The mortality of HIV/AIDS until today is around 90% and the average time from infection to death is approximately 8–10 years. If the HIV infection is continued to AIDS, the life expectancy of the patient is only up to two years.[5] Therefore, better knowledge is required to fight this chronic disease.[6]

The only way to avoid patients from mortality at this moment is to control viral load and the cluster od differentiation 4+ (CD4+) levels increase.[7] Hence, factors that affect the viral load and CD4+ on the disease should be well explored. Toll-like receptors (TLR), encoded by the TLR gene, might play a role as a pattern recognition receptor (PRR).[8] The TLR-9 which is located in the endosome recognizes nucleic acids, including the genome of HIV.[9] TLR-9 gene is located on chromosome 3p21.3, and especially the polymorphism at G1174A has been widely investigated concerning HIV/AIDS.[10,11] HIV patients with

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Table 1 Clinical Characteristic of HIV/AIDS Patients in West Java

	Total (n=87)	Proportion (%)
Gender (n= 87)		
Male	73	83.9
Female	14	16.1
Injected Drug Users (IDU) (n=87)		
IDU	53	60.9
Non-IDU	34	39.1
Tuberculosis (n=85)		
Tuberculosis	4	4.7
Non-Tuberculosis	81	95.3

TLR-9 gene polymorphisms have an increase in viral load and a decrease in CD4+.[10] This study aimed to explore the proportion of the TLR-9 gene polymorphisms at rs352139 among HIV/AIDS patients.

Methods

The design of this study was a retrospective descriptive cross-sectional study. A total of 96 HIV/AIDS patients were recruited from West Java in 2013 of whom the injecting drug use (IDU) community was involved from Garut, Bogor, Tangerang, Depok, Sukabumi, Cianjur, and Bandung.

Blood from the vein was collected in 3 ml EDTA tubes and stored in cold conditions (+4) before being sent to Bandung, West Java, Indonesia, where DNA was isolated according to the manufacturer's protocol (QIAamp DNA Blood Mini Kit, Cat No.51104, Qiagen). Polymorphisms in the TLR-9 gene at rs352139 (G1174A) were examined by the Golden Gate® Genotyping Assay for VeraCode®/ BeadXpressIllumina®. The machine used in this study screened 96 participants in each

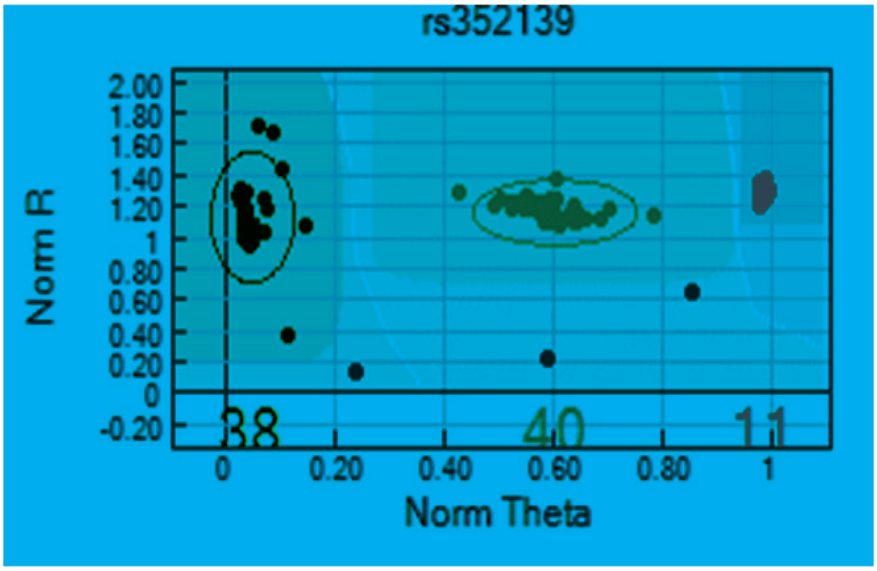

Figure 1 Proportion of Polymorphisms TLR 9 rs352139 in West Java Using Illumina's GenomeStudio®

Note: Each dot represents individual genotypes in one plate for 96 people. The pink area is designated as GG genotype (wild type), the purple area is GA, the blue area is GG, respectively

Table 2 Comparative Frequency Distribution of TLR-9 rs352139 Genotype in Different Populations

Population	Genotype (%)			Allele (%)		Study on disease	P-Value	*P-value	Reference
	GG	GA	AA	G	A				
West Java	42.7	44.9	12.4	65.2	34.8	HIV/AIDS			Present study
Indonesia	11	52	37	37	63	Tuberculosis vs Control	GG (0.00222)	0.17	Kobayashi K, et al.[12]
	12.1	41.6	46.3	32.9	67.1		G(0.0489)		
Vietnam	10.1	45.3	44.6	32.8	67.2	Tuberculosis vs Control	GG (0.239)	0.65	Kobayashi K, et al.[12]
	8.8	40.2	51	28.9	71.1		G(0.127)		
China	20.5	35	35	44.5	65	Tuberculosis vs Control		0.98	Yang Y, et al.[13]
	12.2	53.8	34	41.1	58.9				
Mexico	21.1	53.3	25.6	47.8	52.2	Tuberculosis vs Control	GG (0.01)	0.73	Torres-García D, et al.[14]

plate and detect up to 48 SNPs. In short, DNA was activated to bind paramagnetic samples, and hybridization was followed according to the manufacturer's protocol. The microbead code was identified and a fluorescent signal was detected (BeadXpress® Reader). During the scan, the laser beam penetrated the digital writing to produce unique codes. Data were generated and analyzed (Illumina's GenomeStudio®). The distribution of each allele and genotype of each number was counted for frequency and compared with published global frequencies.

The study protocol was approved by the Research Ethics Committee of Universitas Padjadjaran, Bandung, Indonesia (no. 949/UN6.KEP/EC/2018). The data was presented in proportion.

Results

In total, 96 participants of IDUers from the West Java area were included in this study. However, only 89 participants had complete data of genotyping, and only 87 participants had complete clinical data, consisting of 73 males (83.9%) and 14 females (16.1%). Among them, there were 53 participants (60.9%) who regularly used injected drugs and 4 participants (4.7%) who had tuberculosis (Table 1). The mean age among HIV patients was 30 years old (SD +/- 6.1 years).

As expected, the mean CD4+ count among HIV patients was low which was 318.02 mm3 (SD ± 273.1). The normal CD4+ count was 1500 mm^3. The proportion of rs352139 showed the genotype frequency of GG, GA, and AA as 42.7%, 44.9%, and 12.4%, resulting in a total proportion nucleotide change of 57.3% (Figure 1). The allele proportion of G was 65.2% and for A was 34.8%.

Discussion

AIDS is an immunosuppressive condition caused by HIV. The HIV/AIDS is closely related to various opportunistic infections, secondary neoplasms, and certain neurologic manifestations due to HIV infection. The virus targets CD4+ cells and thus CD4+ levels below 200 cells/ul are associated with HIV infection that later develops into AIDS.[15]

The TLR-9 is a pattern-recognition receptor (PRRs) which is innate immune response that play a role in the first defense against the virus.[9] Polymorphisms in TLR-9 have an important role in the progression of HIV/AIDS; it increases the clinical progression of the disease.[10] To better knowledge of TLR-9 polymorphisms, the proportion of the TLR-9 gene among HIV/ AIDS patients should be revealed. A study by Skevakiet al.[10] has shown that there are two SNPs in the TLR-9 gene that play a key role in nucleotide change at rs352140 or A1635G, and at rs352139 G1174A, as those polymorphisms have a role in HIV/AIDS clinical progression.[10] Since only one polymorphisms data available in our study, we can only describe the proportion of TLR-9 rs352139 gene among HIV/AIDS patient in West Java, Indonesia

The proportion TLR-9 rs352139 gene polymorphisms among HIV/AIDS patient has not been reported in many studies,

only one data from the previous study that represent the frequency of this gene. However, studies on Tuberculosis exploring TLR-9 rs352139 gene polymorphisms have shown genotype frequency variation in different populations as shown in Table 2. The total proportion of polymorphisms in our study has shown a similar result from published data (46.42%)[10], suggesting that TLR-9 rs352139 polymorphisms might have a role in HIV/AIDS rapid progression. However, the prevalence of the TLR-9 rs352139 gene has been shown differently with several tuberculosis studies in Vietnam, China, and Indonesia, and Mexico (Table 2).

TLR-9 single nucleotide polymorphism (SNP) at rs352139 is located in the intronic region of TLR-9. Introns do not play a role in DNA synthesis. Interestingly, in TLR-9 polymorphisms, the intron region might influence the HIV/AIDS disease. The presence of G and C alleles is associated with the down regulation of TLR-9 expression at the transcription level. Conversely, the presence of allele A induces TLR-9 regulation.[14] The mechanism of the interaction between HIV-1 and TLR-9 polymorphisms remained poorly understood. Several hypotheses have been described; one of the hypotheses is when the reverse transcription phase in viral replication, HIV-1 producing dsDNA containing CpG motives. This is transferred from the cytoplasm to the nucleus and integrated into the host cell genome. Since TLR-9 is located in endosomal cells, debris that is phagocyted from HIV-1 cells, contain pro-viral DNA that activates macrophages through TLR-9. The activated TLR induces the production of proinflammatory cytokines and modulates viral DNA who integrated into the cells genome to replicate. This process causes HIV replication to increase and will decrease in CD4 + levels. TLR-9 stimulation via CpG also induces IP10 production from plasmacytoid dendritic cells, monocytes, and B cells. IP10 is elevated in HIV infections and associated with immune activation, plasma viral load, and CD4 decline.[16,17] TLR-9 can also be stimulated by CpG DNA from bacteria and microbial translocation and plasma levels from bacterial DNA both increase in HIV patients and correlate with immune activation.[17] The CpG genome of HIV plays a role in the latency of the disease.[18] Normally, without polymorphisms, TLR-9 will recognize and or target the CpG genome of HIV, thereby reducing the level of CpG, but on the polymorphisms of TLR-9, this doesnot happen.[19] From the hypothesis, these polymorphisms will make a rapid clinical progression of the disease.

The study was only able to detect one type of polymorphism, which is rs352139. The complete data of TLR-9 polymorphisms and correlation study between the TLR-9 rs352139 gene and HIV/AIDS is needed in the future study. The distribution of TLR-9 polymorphisms in the larger population in West Java has not been conducted. Therefore, further studies need to include a control group.

In conclusion, total polymorphisms in our study are higher than the wild type and shows similar results from the previous study in Mexico and NCBI gene bank. However, the proportion is different from other study in Indonesia, Vietnam, and China. A further cohort study is of great interest to associate the rs352139 polymorphisms with a decrease of CD4+cells in HIV/AIDS patients, confirming a rapid disease progression.

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