Distribution of VDR Gene Polymorphisms \(Bsm-I\) rs1544410 and \(Apa-I\) rs7975232 among HIV/AIDS Patients from West Java

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Abstract

Vitamin D receptor, encoded by VDR gene, mediates vitamin D functions by not only regulating calcium metabolism and homeostasis but also in regulating immune response. Polymorphisms in VDR gene may increase the progression of human immunodeficiency virus (HIV) infection into acquired immunodeficiency syndrome (AIDS). This study aimed to explore the distribution of VDR polymorphisms among HIV sero-positive patients in West Java. A cross-sectional study was performed, recruiting 96 patients infected with HIV and VDR polymorphisms were analyzed. The genotype distributions of \(Bsm-I\) among HIV-infected patients were 2.2%, 18.5%, and 79.3% for BB, Bb, and bb, respectively whereas the distributions of \(Apa-I\) were 54.4%, 38.9%, and 6.7% for AA, Aa and aa, respectively. The frequency of VDR polymorphisms in \(Bsm-I\) among HIV-infected patients in West Java were considered high for b allele (88.6%), and in contrast for A allele in \(Apa-I\) that was 73.91%. Further studies involving healthy controls are needed to explore the VDR polymorphisms distribution in general population. Moreover, a cohort study, albeit challenging, is needed to further assess the association between VDR polymorphisms and the progression of HIV infection.

Key words: \(Apa-I\), \(Bsm-I\), polymorphism, vitamin D Receptor, VDR gene

Distribusi Polimorfisme gen VDR \(Bsm-I\) rs1544410 dan \(Apa-I\) rs7975232 pada Pasien HIV/AIDS di Jawa Barat

Abstrak

Reseptor vitamin D yang dikode oleh gen VDR mempunyai peranan penting terhadap fungsi vitamin D; tidak hanya dalam regulasi metabolisme dan keseimbangan kalsium namun juga berperan dalam mereguasi respons imun. Polimorfisme pada gen VDR dapat meningkatkan perkembangan infeksi human immunodeficiency virus (HIV) menjadi acquired immunodeficiency syndrome (AIDS). Penelitian ini bertujuan mengetahui distribusi polimorfisme gen VDR pada pasien HIV di Jawa Barat. Penelitian ini melibatkan 96 pasien HIV dan dilakukan analisis polimorfisme gen VDR. Distribusi genotip \(Bsm-I\) pada pasien HIV di Jawa Barat adalah 2,2%, 18,5%, dan 79,3% untuk BB, Bb, dan bb, secara berurutan; sedangkan pada \(Apa-I\) adalah 54,4%, 38,9%, dan 6,7% untuk AA, Aa, dan aa. Frekuensi polimorfisme pada \(Bsm-I\) pada pasien HIV di Jawa Barat tergolong tinggi pada alel b (88,6%), dan berbanding terbalik pada dan \(Apa-I\) dengan alel A yaitu 73,91%. Penelitian lebih lanjut yang melibatkan individu kontrol diperlukan untuk mengetahui distribusi polimorfisme gen VDR pada populasi umum. Selain itu, studi kohort pada pasien HIV/AIDS diperlukan untuk menilai hubungan antara polimorfisme gen VDR terhadap progresivitas infeksi HIV.

Kata kunci: \(Apa-I\), \(Bsm-I\), polimorfisme, reseptor vitamin D, VDR

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**Introduction**

Acquired immune deficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV) infection, is a syndrome associated with an increased risk of opportunistic infection due to an impaired immune system. The high prevalence of HIV/AIDS is still a vast global health issue, where Indonesia accounts for approximately 620,000 cases.\(^1,2,3\)

HIV infection is a chronic inflammatory disease characterized by Th1-like response. Therefore, the susceptibility to HIV infection and the rates of AIDS as a disease progression are associated with the host immune response.\(^1\) Vitamin D\(_3\), (1,25(OH))\(_2\)D\(_3\) is essential in the immune system modulation.\(^4\) Vitamin D interacts with its specific intracellular vitamin D receptor and forms a complex Retinoid X Receptor (RXR) that further mediates vitamin D functions.\(^5\) The role of vitamin D\(_3\) - VDR complex in the immune system is to inhibit the maturation of dendritic cells as well as the proliferation of lymphocyte in promoting proinflammatory cytokine synthesis and immune response. The inhibiting effect of vitamin D on the immune response appears to target Th1 cells by preventing their activation and lymphokine production. Furthermore, vitamin D triggers antimicrobial pathways in host cells and activates genes that enhance the immunity.\(^6\)

Vitamin D\(_3\) also plays a role in mineral metabolism, especially in regulating the calcium and skeletal homeostasis. Thus, the deficiency of vitamin D is related to skeletal diseases.\(^7\) Non-skeletal diseases such as cancer, diabetes, cardiovascular diseases, and autoimmunity may also be influenced by the deficiency of vitamin D.\(^8,9\) Vitamin D deficiency is related to a higher risk of infection and poor prognosis of infectious diseases, such as tuberculosis and HIV.\(^10\)

The underlying mechanisms to infection resistance and the variability observed in the rate of disease progression after infection are likely to be multifactorial, which may include the host genetic factor. VDR gene has several single nucleotide polymorphisms (SNPs) such as Bsm-I, Apa-I, and Taq-I that are found in 3' untranslated region (UTR); Cdx2 and A-1012G in 5' regulatory region, and Fok-I in the coding region.\(^5\) Bsm-I polymorphism or rs1544410 is formed by substitution of adenine-guanine (A/G) and consists of B and b allele, whereas Apa-I or 7975232 is created by the substitution of guanine-thymine (G/T) and consists of A and a alleles. Interestingly, polymorphism in the 3’UTR VDR gene is associated with the mRNA stability of VDR that adjusts the expression of VDR and other polymorphism in VDR which also shows a modulation in vitamin D-VDR complex activity.\(^5\)

VDR gene, which encodes vitamin D receptor, has various polymorphisms and the variations at the VDR locus have been associated with the susceptibility and progression to several immune diseases.\(^5\) VDR polymorphism has shown to play a significant role in the susceptibility of other diseases, such as spin\(^17\) and gynecological carcinoma.\(^8\) As for polymorphisms in VDR gene Fok-I, infected HIV patients carrying the Ff genotype has been considered prone to a faster progression to AIDS.\(^11\)

The distribution of VDR polymorphisms differ across the global population. In Brazil, for example, the Bsm-I genotype BB, Bb, and bb are found in 16.5%, 50.4%, and 33.1%, respectively. However, in China, only BB (15.8%) and bb (84.2%) polymorphisms are observed.\(^12,13\) In Indonesia, the VDR polymorphism has been studied in several populations including, among others, in Batak ethnic in North Sumatera.\(^14\) The difference of VDR polymorphisms in various populations has led this study to explore the distribution of VDR gene polymorphisms among HIV seropositive patients in West Java. The genotype carried by the HIV seropositive patients may serve as a predictor to consider whether an individual is prone to a faster progression to AIDS.

**Methods**

This was a descriptive cross-sectional study on a total of 96 HIV/AIDS patients recruited from several cities in West Java in 2013. In brief, upon informed consent, genomic DNA was extracted according to the manufacturer’s procedure. Single nucleotide polymorphisms of the VDR gene at rs1544410, rs7975232, rs731236 and rs 2228570 or at the restriction site of Bsm-I, Apa-I, Taq-I, and Fok-I, respectively, were analyzed (Bead Xpress Reader; Illumina\(^9\)) at the Molecular Biology Laboratory of Faculty of Medicine, Universitas Padjadjaran. This genotyping system machine is able to examine a total of 48 Single Nucleotide Polymorphisms (SNPs) of the four SNPs of the VDR gene examined. In brief, DNA was activated to bind to paramagnetic samples, and hybridization was followed according to the manufacturer’s protocol. Microbead code was then identified and the fluorescent signal was detected. The distribution of VDR gene

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polymorphisms was visualized in a single dot that represented individual genotype. Data generated were then analyzed (GenomeStudio®, Illumina®). The distribution of VDR gene polymorphisms was counted for the frequency and presented in the table.

This study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran Bandung through the issuance of the ethical clearance no. 948/UN6.KEP/EC/2018.

Results

In total, 96 HIV/AIDS patients were initially recruited. However, data on genotype could only be retrieved from 87 patients, consisting of 73 males (83.9%) and 14 females (16.1%). The mean age of the patients was 30.2 years old (SD±6.1). These patients were mostly (60.9%) injecting drug users (IDUs) and tuberculosis co-infection was only found in a small proportion (4.7%) of the HIV/AIDS patients as depicted in Table 1. From the medical record collected, the mean of CD4 count was identified to be 318.02 (SD±273.1). Since this was a cross-sectional study, the CD4 count could not be analyzed further for the correlation with the VDR polymorphisms result below.

The distribution of the VDR gene polymorphisms was visualized in a single dot that represented individual genotype as shown in Figures 1 and 2. Only Bsm-I and Apa-I polymorphisms were detected whereas polymorphisms of Taq-I and Fok-I were failed to be detected due to some technical error. The genotype of Bsm-I polymorphism was mostly bb (79.3%), with the most frequent b allele of 88.6%. Meanwhile, the genotype of Apa-I polymorphism was mostly AA (54.4%), with allele A as the most prevalent (73.9%) as depicted in Table 2.

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

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>73</td>
<td>83.9</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>16.1</td>
</tr>
<tr>
<td>Risk Factor</td>
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<tr>
<td>IDU</td>
<td>53</td>
<td>60.9</td>
</tr>
<tr>
<td>Non-IDU</td>
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<td>39.1</td>
</tr>
<tr>
<td>TB History</td>
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<td></td>
</tr>
<tr>
<td>TB</td>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td>No TB history</td>
<td>81</td>
<td>93.1</td>
</tr>
<tr>
<td>Missing data</td>
<td>2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Notes: IDU: injection drug use; TB: tuberculosis

Table 2 Distribution of Genotype and Allele of VDR Polymorphisms at Restriction Site of Bsm-I and Apa-I in HIV/AIDS Patients from West Java

<table>
<thead>
<tr>
<th>VDR polymorphisms</th>
<th>Genotype (%)</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bsm-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>2 (2.2)</td>
<td>B</td>
</tr>
<tr>
<td>Bb</td>
<td>17 (18.5)</td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>73 (79.3)</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apa-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>49 (54.4)</td>
<td>A</td>
</tr>
<tr>
<td>Aa</td>
<td>35 (38.9)</td>
<td>a</td>
</tr>
<tr>
<td>aa</td>
<td>6 (6.7)</td>
<td></td>
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</tbody>
</table>
Vitamin D receptor, encoded by the VDR gene, is a nuclear receptor (NR) superfamily that has an essential function in regulating the immune system in macrophages, dendritic cells, neutrophils, B cells, natural killer (NK) cells, and T lymphocyte. Studies on VDR polymorphisms have shown a significant role in susceptibility to HIV and an increasing role in the progression of HIV infection to AIDS, both in adult and pediatric patients. The VDR polymorphisms across the global population have different distributions. The present study in West Java has described the distribution of VDR gene polymorphisms at rs1544410 and rs7975232 or at the restriction site of Bsm-I and Apa-I, respectively. The result in Bsm-I polymorphism in Sundanese HIV infected patients has shown a high portion of b allele (79.3%), similar to the study on the general population of Batak people, which is another ethnic in Indonesia. The distribution of Bsm-I allele b is also in agreement with that of other Asian population but differs from the population from other continents in South America. It seems that the high portion of the b allele in the HIV-infected patients in this study also reflects the general population distribution and, thus, shows no difference. The important role of having a b allele needs to be further analyzed, for example to assess its association with the CD4 count.

Lymphocyte T CD4 has a gp120 glycoprotein structure that is associated with HIV initial infection. HIV binds with gp120 to begin its invasion and it will eventually damage the CD4 cells. CD4 cell death, caused by HIV, is associated with caspase-3-mediated apoptosis and caspase-1-mediated pyroptosis. Thus, individuals with VDR polymorphisms may have lower CD4 counts due to the more rapid proliferation of the virus. Interestingly, a previous study of VDR SNPs related to HIV infection has shown that Bsm-I and Apa-I polymorphisms have produced lower IL-10 and had a decreased lymphocyte response. IL-10 function as an inhibitor for HIV-1 proliferation.

In contrast, the Apa-I allele in Sundanese is mostly A (54.4%), which is equally distributed with allele a, and is similar to other countries in Asia, including in China and India. Again, the important result of having allele a needs further investigation. Furthermore, due to a technical problem in the laboratory, this study has failed to detect Taq-I and Fok-I polymorphisms. Fok-I polymorphism has been known to have a significant association with HIV/AIDS progression. CD4 cell count drops to below 200/uL faster in HIV infected patients with Fok-1 polymorphism or Ff carriers; therefore, the measurement of regular CD4 cell count is necessary to monitor the progression of HIV infection to AIDS when HIV-infection individual is carrying Ff genotype. Moreover, VDR polymorphisms also increase phagocytosis of macrophage towards M.tuberculosis that it may affect the progression of HIV-TB patients.

VDR gene polymorphisms may have impacted in vitamin D absorption among HIV/AIDS patients and may of great interest to explore further. Vitamin D supplementation in patients infected with HIV can improve antibacterial immunity. Vitamin D is able to inhibit the proliferation of T lymphocyte. Therefore, by giving HIV/AIDS patients vitamin D supplementation, a protective effect may be gained due to decreasing activated immune system and HIV target cells.

This study has encountered several limitations because it was only performed among HIV/AIDS patients that the comparison in the distribution of VDR polymorphism, especially Bsm-I and Apa-I, in the general population cannot be observed and cannot be compared with healthy controls. Therefore, studies involving healthy controls in the same area are necessary. Furthermore, a cohort study is considered a better study design to explore the progression of HIV infection to...
AIDS or to assess the fall of CD4 cell count as an effect of VDR polymorphisms.

To conclude, the frequency of VDR polymorphisms among HIV-infected patients in West Java is considered high for b allele of Bsm-I (88.6%) and a allele of Apa-I is common (26.1%). VDR polymorphisms among HIV-infected patients may have a predictor value in the progression of HIV infection.

References