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ORIGINAL ARTICLE

HIV-1 drug resistance-associated mutations in relation to viral load among HIV/AIDS patients at Dr. M. Djamil-Hospital Padang

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ABSTRACT

BACKGROUND
According to the WHO, 38 million people suffer from HIV worldwide and according to the HIV Drug Resistance Report, the prevalence of antiretroviral therapy (ART) resistance is 3-29%. Drug resistance-associated mutations (DRAMs) are the presence of one or more HIV mutations that reduce the ability of certain drugs to inhibit viral replication and that will increase viral replication and HIV RNA, which can lead to therapeutic failure. The objective of this study was to determine the prevalence of HIV-1 DRAMs among patients with chronic HIV-1 infections and to compare HIV RNA viral load between M184V and K103N mutations.

METHODS
A cross-sectional was conducted involving 80 patients with HIV who met the inclusion criteria. The study subjects were examined for genotype and HIV RNA viral load, both using the polymerase chain reaction (PCR). Data were analyzed with the Kruskal-Wallis test.

RESULTS
The overall drug resistance mutation prevalence was 10.0%. The most common mutations were M184V and K103N. There was a significant difference between the median HIV RNA viral load counts in patients with either M184V or K103N, and with both M184V and K103N mutations, the values being 45.420, 13.207, and 97.517 copies/mL, respectively ($p<0.001$).

CONCLUSION
The HIV RNA viral load count was higher in the mutation group than in the group without mutation. Long-term and ongoing surveillance of HIV DRAMs among these patients is necessary, which will help us to adjust the treatment regimen.

Keywords : Drug resistance mutation, HIV RNA, viral load

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a global health issue. According to data from the World Health Organization (WHO) it is estimated that there are 38 million people suffering from the Human Immunodeficiency Virus (HIV) worldwide with 1.7 million new cases and 690,000 deaths annually. The first case of HIV infection in Indonesia was found in 1987. The number of cases of HIV infection in Indonesia is estimated to continue to grow every year. It is estimated that there were 543,100 Indonesians with HIV infection in 2020. The province of West Sumatra ranks 8th nationally in cumulative cases of HIV infection and AIDS in Indonesia. In 2019, cumulative cases of HIV and AIDS that were recorded in West Sumatra totaled 3,338 HIV cases and 2,087 AIDS cases.[1,2]

The WHO and the United Nations Program on HIV/AIDS (UNAIDS) have set a goal to end the AIDS pandemic as a health threat by 2030 by ensuring that 90% of people infected with HIV are aware of their condition, that 90% of people with HIV receive antiretroviral therapy, and that HIV suppression is achieved in 90% of people receiving antiretroviral therapy (ART). To achieve these targets, virological monitoring and HIV genotypic resistance testing of individuals on ART are necessary as recommended by the WHO.[3] In Dr. M.Djamil central general hospital, Padang, the combinations of first-line ART drugs in use were tenofovir (TDF)/Zidovudine (AZT) or emtricitabine (FTC)/lamivudine (3TC) with Nevirapine (NVP)/Efavirenz (EFV). The use of combination antiretroviral therapy has been shown to control and reduce the progression of viral infections and increase life expectancy. However, therapeutic failures still occur, leading to resistance to ART. Mutation of the virus will result in the mutated virus multiplying itself, thus increasing the amount of circulating HIV RNA in the blood and causing therapeutic failure.[4-6]

One of the methods to determine the presence of viral resistance is to identify drug resistance-associated mutations (DRAMs) in HIV-1, especially in the genes encoding for reverse transcriptase and protease inhibitors, which are the targets of ART. The method that can be used is genotyping, which consists of amplification of genetic material using polymerase chain reaction (PCR) and nucleotide sequencing. Genotyping is the gold standard method for identifying DRAMs and is recommended in patients with therapeutic failure after ART administration, to determine further therapeutic options.[7]

According to the WHO, pretreatment HIV drug resistance (HIVDR) to non-nucleoside reverse-transcriptase inhibitors (NNRTI) can affect more than 10% of adults starting therapy and is found up to 3 times more often in people with previous exposure to antiretroviral drugs.[8] Zuo et al.[9] reported that the M184V mutation causes resistance to lamivudine and that K103N is the most frequent mutation in nearly all NNRTI drugs. Maruapula et al. [10] reported that there were one or more mutations in patients who failed first line therapy, with M184V and K103N being the most common. Drug resistance (DR) among HIV should receive more attention. First, the amplification of HIV gene segments in HIV samples is usually technically challenging. [11] One study investigated the HIV-1 subtype classification and the prevalence of drug resistance mutations (DRMs) in ART-experienced and ART-naïve residents of Pontianak, West Kalimantan, Indonesia, showing that acquired drug resistance (ADR) was found in 28.5% of ART-experienced individuals.[12] Another study by Hutapea et al.[13] reported that the prevalence of ART resistance in Papua was 6.3%. The AIDs Clinical Trial Group (ACTG) A5230 study reported a prevalence of drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs) was 19%.[14] In addition, DR detection is still expensive, resulting in an unknown DR status in HIV patients who are not covered by the free DR monitoring program. Data on the prevalence of DR or DRM are limited in Indonesia.

In this study, we aimed to investigate the prevalence of HIV-1 DRAMs among patients with chronic HIV-1 infections and to determine the relation between DRM and HIV RNA viral load, as an alternative to DRM tests for estimating HIV resistance associated mutations and viral load among HIV/AIDS patients.

METHODS

Research design

This was an analytical observational study with a cross-sectional approach conducted at the Voluntary Counseling and Test (VCT) Internal Medicine Clinic at Dr. M. Djamil Hospital, Padang. The blood samples from the subjects were collected from January to June 2022.

Research subjects

From January to June 2022, a total of 80 subjects who had received NRTI and non-nucleoside reverse transcriptase inhibitor (NNRTI) therapy for more than 6 months were included into the study. A study reported a prevalence of drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs) of 19%. [14]. Subjects who met the inclusion criteria and were not included in the exclusion criteria comprised the study sample. The inclusion criteria were patients over 18 years of age, who had received NRTI and non-nucleoside reverse transcriptase inhibitor (NNRTI) therapy for more than 6 months and were willing to participate in the study. The exclusion criteria were patients coinfected with hepatitis B and hepatitis C, active pulmonary tuberculosis cases, and/or cases not receiving anti-tuberculosis treatment, patients with autoimmune disease, and patients on radiotherapy or chemotherapy.

Laboratory procedures

The mutation testing was carried out by means of PCR. The reagents used for extraction were the QIAamp Viral RNA Mini Kit reagent (Cat. no. 52904), while the first and second round PCR reagents used were Qiagen OneStep PCR. The process was carried out in 2 stages, i.e. amplification and sequencing. The HIV RNA testing by PCR method was carried out in the laboratory of Dr. M Djamil Hospital, Padang. The basis of this method is repeated amplification or duplication of the target sequence or nucleotide sequences.

Statistical analysis

HIVDRM counts were the primary outcome of concern. The predictor variables included age, sex, viral load, treatment period, ART regimen, and HIV-1 subtype. Before evaluating the independent variable, we carried out normality tests to determine the subsequent parametric test. Based on the Kolmogorov-Smirnov test, the data on HIV RNA were not normally distributed, thus the Kruskal-Wallis test was used to compare the medians of mutated HIV RNA viral loads and a p-value of less than 0.05 was considered statistically significant.

Ethical considerations

This study was approved by the Health Research Ethics Committee of Dr. M. Djamil Hospital under No. 440/KPK/2021.

RESULTS

Characteristics of the study subjects

Table 1 shows the characteristics of the study subjects. This study involved 80 subjects, 74 male (92.5%) and 6 female (7.5%), who had a mean age of 35.5 ± 9.1 years and were divided into age groups, with the majority of the subjects being in the age group of 31–40 years. This study showed that the longest treatment time was 168 months and the shortest time was 6 months, the average length of treatment obtained being 43.3 months. All patients received first-line ART therapy. The first-line ART combinations used were TDF+FTC+EFV in 47 (58.8%) patients, TDF+3TC+EFV in 9 (11.3%) patients, AZT+3TC+NVP in 17 (21.3%) patients, and AZT+3TC+EFV in 7 (8.8%) patients. This study found that 32 (40.0%) of the subjects had CD4 counts below 250/mm^3 and that 48 patients (60.0%) had CD4 counts above 250/mm^3. The mean CD4 count in this study was 298.6 ± 172.68 cells/mm^3 (Table 1).

Table 1. Demographics and laboratory data of study patients (n=80)

Variable	n (%)
Gender	
Male	74 (92.50)
Female	6 (7.50)
Age (years)	35.90 ± 9.18
≤30	27 (33.75)
31-40	34 (42.50)
41-50	13 (16.25)
>50	6 (7.50)
Length of treatment (months)	43.30±30.40
6-24	27 (33.80)
>24	53 (66.20)
ART regimen	
TDF+FTC+EFV	47 (58.80)
TDF+3TC+EFV	17 (11.30)
AZT+3TC+NVP	9 (21.30)
AZT+3TC+EFV	7 (8.80)
CD4 count (cells/mm ^{3})	298.1±172.6
≤250	32 (40.00)
>250	48 (60.00)

Note: Data presented as n (%), except for age, length of treatment and CD4 count as mean±SD
*3TC: Lamivudine, ART: Antiretroviral Therapy, AZT: Zidovudine, EFV: Efavirenz, FTC: Emtricitabine, NVP: Nevirapine, TDF: Tenofovir.

Drug resistance-associated mutations

The HIV reverse transcriptase (RT) gene sequence of this study showed mutations in 8 (10%) of the study subjects and none in 72 (90%) (Table 2). The M184V mutation was found in 4 (5%) of the patients, the K103N mutation in 1 (1.25%) patient, and both M184V and K103N mutations were found in 3 (3.75%) patients. Table 3 presents the differences in HIV RNA viral load (copies/mL) between patients with and without mutations. There was a significant difference in median HIV RNA viral load between patients with mutations (163.0 copies/mL) and patients without mutations (60.92 copies/mL) ($p<0.001$) (Table 3).

Virological failure

Because the HIV RNA data were not normally distributed according to the the Kolmogorov-Smirnov test, the Kruskal-Wallis test was carried out. There was a statistically significant difference between the median HIV RNA viral loads in patients with no mutation (163.00 copies/mL), with the M184 mutation (45.42 copies/mL), with the K 103N mutation (13.207 copies/mL) and with both M184V and K103 mutations (97.51 copies/mL) ($p<0.001$) (Table 4).

Table 2. M184V and K103N mutations in the study subjects

Variable	n (%)
No mutations	72 (90.00)
Mutation present	
M184V mutation	4 (5.00)
K103N mutation	1 (1.25)
M184V and K103N mutations	3 (3.75)

DISCUSSION

In 80 patients receiving ART in the M. Djamil central general hospital, the mutations were found in 8 patients. All patients received one combination of first line ART**:** TDF+FTC+EFV, TDF+3TC+EFV, AZT+3TC+NVP, or AZT+3TC+EFV. The overall mutation prevalence was 10% of all patients. M184V mutations occurred in four patients, K103N mutations in one patient, and both mutations in three patients.

M184V and K103N mutations were the most common. M184V is an NRTI mutation which induces high level resistance to 3TC, while K103N is an NNRTI mutation that gives rise to high level resistance to nevirapine and variable resistance to efavirenz. A similar mutation pattern was reported in another study by Al-Omairi et al.[15] in Oman. They found that M184V/I, K103N/S, and G190A/S/E were the most common mutations detected among 98 patients. They also found that the mutations had been selected by the previous HIV drug exposures, and that the most common HIV drugs used prior to the resistance tests were lamivudine, zidovudine, and efavirenz.

In our study, mutations occurred in patients who had undergone treatment between 12-76 months. The correlation between duration of treatment and emergence of mutations could not be determined, but according to Scriven et al.[16], longer treatment duration is associated with drug resistance mutation. Seu et al.[17] in Zambia found that the treatment duration up to the emergence of first ART resistance (in which it was examined through resistance) was between 1.7-4.7 years with an average of 3.2 years. However, these reports have not yet given rise to a consensus on the relationship between HIV drug-resistance associated mutations and viral load among HIV/AIDS patients, such that a more recent study is still needed.

Table 3. Differences in HIV RNA viral load between mutations and no mutations

Variable	n	Median HIV RNA (copies/mL)	p value
No mutations	72	163.0	$p < 0.001$
Mutations present	8	60.92	

Table 4. Differences in HIV RNA viral load between M184V and K103N mutations

Variable	n	Median (copies/mL)	p value
No mutations	72	163.0	$p < 0.001$
M184V mutation	4	45.420	
K103N mutation	1	13.207	
M184V and K103N mutations	3	97.517	

Even though the percentage of patients who did not have mutations was still much greater (88.88%) than that of patients who had, the latter should not be underestimated because these mutations can cause resistance to various lines of ART treatment and even cause ART cross-resistance resulting in therapeutic failure. The emergence of various mutations in the genes that cause ART resistance can lead to accelerated HIV development and transmission of drug-resistant strains between susceptible individuals. In one study, the magnitude of first-line ART treatment failure based on virological criteria was 20.85%.[18] ART failure develops in about 20% of patients with HIV receiving first-line ART in developing countries with low resources.[19] The WHO projects that this number will continue to increase over the next 10 years.[20]

Statistically, there was a significant difference between the amount of HIV RNA in patients with M184V and K103N mutations compared to patients without these mutations. Our study found that 7 out of 8 patients who had the mutations had a viral load above 1000 copies/mL. This is in line with a study by Hutapea et al.[21] in Papua which also found a statistically significant relationship between the amount of HIV RNA and mutations. It was found that patients with HIV RNA values of >1000 copies/mL had a higher probability of mutations compared to patients with HIV RNA values below 1000 copies/mL. Our study found that one patient with DRM had an HIV RNA viral load below 1000 copies/mL, the cause of which has not been investigated.

The primary limitation to the generalization of these results was the limited sample size where all data were determined in a one-time collected sample. This factor might limit the ability to generalize to national prevalence. Further national and regional surveillance are still needed. Second, this study also did not take into account other factors that may result in mutations, such as patient adherence to therapy and disease stage when starting therapy.

CONCLUSIONS

The prevalence of drug resistance-associated mutations was 10.0%. The HIV RNA viral load count was lower in the mutation group than in the group without mutations. HIV genotypic assays before ART initiation in patients with chronic HIV-1 infection should be considered before starting therapy.

Conflict of Interest

Competing interests: No relevant disclosures.

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Author Contributions

RN wrote the manuscript and designed the experiments; DE collected samples and clinical information; R analyzed the data; DE and R made manuscript revisions.
All authors have read and approved the final manuscript.

Data Availability Statement

Requests for the original data presented in this study can be directed to the corresponding author.

Declaration of Use of AI in Scientific Writing

Nothing to declare

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