Prevalence of drug-resistant mutation among drug-treated HIV/AIDS inpatient in Airlangga University teaching hospital, Surabaya, Indonesia

B E Rachman[1,2*], S Q Khairunisa[3], A M Witaningrum[3], M Q Yunifiar[3], P Widiyanti[3,4] and Nasronudin[1,2]

[1]Airlangga University Hospital, Surabaya, Indonesia
[2]Faculty of Medicine, Airlangga University, Surabaya, Indonesia,
[3]Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia
[4]Biomedical Engineering, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia
[*]Corresponding author: drbrianer@gmail.com

Abstract. Increased use of antiretroviral therapy did not completely reduce the incidence of HIV/AIDShospitalization. Various factors can be involved. The aim of this study is to examine HIV-1 drug resistance mutations profile in drug-treated HIV/AIDS patients who underwent hospitalization. HIV/AIDS patients who are admitted to hospital who had received ART are included in the study and then examined for the presence of drug resistance-associated mutations. A total of 17 samples were included in the study, but only 11 samples that could be sequence analyzed. On the mutation examination of drug resistance in reverse transcriptase gene, it werefound a major mutation in K103N (9%) and G190A (9%). Most minor mutations were found in A98S (18.1%), followed by M41L, M184V, L210W, T215Y, V108l, Y181C and H221Y at 9% each. Whereas, on examination of drug resistance mutations in protease genes, there is a major mutation in I84V of 9%. Most minor mutations on M36I (45.4%), followed by L10I (36.3%), H69K (36.3%), I93L (27.2%), G16E, L89M, K20R 18.1%, L64V and V771I 9% respectively.A large number of mutated samples pose a challenge in long-term antiretroviral treatment, so a breakthrough policy is needed to minimize the impact.

1. Introduction

Human Immunodeficiency Virus/ Acquired Immuno Deficiency Syndrome (HIV/AIDS) is still one of the most remarkable health challenges in the world as well as in Indonesia. About 36.7 million people are infected with HIV, and millions of people have died of AIDS since the beginning of the epidemic. To date, there has been an increase in the number of people infected with HIV from 33 million in 2010 to 36 million by 2016.[1] In Indonesia, the number of people living with AIDS was cumulatively estimated to be 86.780 at the end of 2016, and as many as 41,250 were newly infected with HIV by 2016.[2] This increase in numbers could result from new infections and increased life expectancy due to antiretroviral therapy scale-up. However, this raises new issues related to the effectiveness of antiretroviral therapy, namely the problem of viral mutation and resistance to antiretroviral therapy. Decreased effectiveness of antiretroviral therapy was reported in several studies related to the presence of HIV-1 drug resistance.[3,4]Several studies have reported HIV-1 mutations in naive HIV/AIDS patients at various sites in Indonesia.[5,6] Other studies have reported HIV-1 mutations in

HIV / AIDS patients who have been receiving antiretroviral therapy (ART) in an outpatient setting.[7]However, there is enoughinformation in Indonesia regarding HIV-1 mutations in patients who have received ART in hospitalization settings. The aim of this study is to examine HIV-1 drug resistance mutations profile in drug-treated HIV/AIDS patients who underwent hospitalization.

2. Materials and Methods

Inpatient HIV / AIDS at the Airlangga University Teaching Hospital during the period March to September 2017 and willing to participate in the study are included in the study. Disease and treatment history such as Anti Retroviral Therapy (ART) regimen, duration of ART, side effects, CD4 levels baseline, opportunistic infections, clinical conditions of the patients, and possible routes of HIV-1 transmission are taken from medical record database. This study is approved by the Institutional EthicsCommittees of Airlangga University and Airlangga University Teaching Hospital. Informed consent is obtained from every subject prior to sample collection. Ten milliliters of ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood is collected from each participant. Then plasma is isolated from peripheral blood samples by centrifugation for 10 min at 2,000 rpm. Peripheral blood mononuclear cells (PBMCs) were also isolated by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO). DNA was extracted from PBMCs using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich).

The HIV-1 pol gene encoding protease (PR gene) and reverse transcriptase (RT gene) areamplified from DNAextracted from PBMCs by nested polymerase chain reaction(PCR) using Ex Taq (Takara Bio, Shiga, Japan) and primersets as follows. To amplify the viral PR gene fragment, theprimers DRPRO5 [5'-AGACAGGYTAATTTTTTAGGGA-3'; corresponding to nucleotides (nt) 2074 to 2095 of anHIV-1 reference strain, HXB2 (GenBank accession no.K03455)] and DRPRO2L (5'-TATGGATTTTCAGGCCCAATTTTTGA-3'; nt 2716 to 2691) were used for the first PCR,and the primers DRPRO1M (5'-AGAGCCAACAGCCCCACCAG-3'; nt 2148 to 2167) and DRPRO6 (5'-ACTTTTGGGCCATCCATTCC-3'; nt 2611 to 2592) were used for thenested PCR. In addition, to amplify the viral RT gene, RT1L(5'-ATGATAGGGGGAATTGGAGGTTT-3'; nt 2388 to2410) and GPR2M (5'-GGACTACAGTCYACTTGTCCATG-3'; nt 4402 to 4380) were used for the first PCR and RT7L(5'-GACCTACACCTGTCAACATAATTGG-3'; nt 2485 to2509) and GPR3L (5'-TTAAAATCACTARCCATTGYTCTCC-3'; nt 4309 to 4285) were used for the nested PCR.PCR products are amplified at the end-point dilution of DNAtemplates were subjected to sequencing analysis to examinethe genomic fragment of the major viral population in asample.

Sequencing analysis of the amplified HIV-1 PR and RT genes was carried out using the BigDye Terminator v3.1 Cycle Sequencing kit with an ABI PRISM3500xL genetic analyzer (Applied Biosystems, Foster City, CA), and data were assembled using Genetyx version 10 software (Genetyx, Tokyo, Japan). The full length of the PR gene (297 bp; nt 2253 to 2549) and the N-terminus of the RT gene (762 bp; nt 2550 to 3311) were sequenced and subjected to subsequent analyses. The determination of drug resistance mutations is based on the guidelines published by the International AIDS Society United States (IAS-USA).[8]QuickAlign, which is available on the HIV sequence database website (www.hiv.lanl.gov/), was used to analyze the appearance rate of natural polymorphic amino acid substitutions. HIV-1 subtyping was carried out using the Recombinant Identification Program (RIP) available on the HIV sequence database website (www.hiv.lanl.gov/). Also, neighbor-joining (NJ) trees with the Kimura two-parameter model were constructed using theMEGA6.2 software.[9,10] Bootstrap values (1,000 replicates) for relevant nodes are reported on a representative tree. If one of the PR and RT genes failed to be sequenced, the subtype was assigned based on the other gene. Furthermore, if a discrepancy was detected in the subtype between PR and RT, it was defined as a recombinant of more than two HIV-1 subtypes and circulating recombinant forms (CRFs).

3. Results

A total of 17 samples of hospitalized patients at the Airlangga University Teaching Hospital were included in the study. Demographic characteristics in the study showed that predominantly male (70.6%) with an average age of 43.35 years old. Most of the samples got HIV transmission routes through heterosexual intercourse (47.1%). Most of the samples still use first-line ART, but theirisone patient already used Lopinavir (LPV/r) as second-line ART. The number of secondary infections has a range of 1-6 (Table 1). Only 11 out of 17 samples can be analyzed sequentially, while the rest are not detected. Drug mutations occurred in 7 of the 11 samples, 2 of which experienced drug resistance-associated mutations. On the mutation examination of drug resistance in reverse transcriptase gene, we found a major mutation in K103N (9%) and G190A (9%). Most minor mutation is found in A98S (18.1%), followed by M41L, M184V, L210W, T215Y, V108l, Y181C,and H221Y at 9% each (Table 2). Whereas, on examination of drug resistance mutations in protease genes, there is a major mutation in I84V of 9%. Most minor mutationson M36I (45.4%), followed by L10I (36.3%), H69K (36.3%), I93L (27.2%), G16E, L89M, K20R 18.1%, and L64V and V771I respectively 9 % (Table 3).

Table 1. Demographic characteristics of study subjects.

Characteristics	Value (n = 17)
Age, mean years (SD)	43.35 (13.92)
Sex, n (%)	
Male	12 (70.6)
Female	5 (29.4)
Risk factor for HIV infection, n (%)	
Heterosexual intercourse	8 (47.1)
Homosexual intercourse	3 (17.6)
Intravenous drug user	6 (35.3)
Type of ART used, n (%)	
AZT	11 (64.7)
3TC	14 (82.3)
TDF	5 (29.4)
EFV	4 (23.5)
NVP	11 (64.7)
LPV/r	1 (5.8)
Number of opportunistic infection, mean (Range)	3.11 (1-6)
Mutation, n (%)	
Yes	7 (63.6)
No	4 (36.4)
HIV-1 Subtype, n (%)	
CRF01_AE	9 (81.8)
B	2 (18.2)

Table 2. Drug resistance mutations in reverse transcriptase inhibitor among HIV-1 patients.

Mutation	**Frequency (%)**		
	All (n = 11)	CRF01_AE (n = 9)	B (n = 2)
K103N	1 (9)	1 (11.1)	
G190A	1 (9)	1 (11.1)	
A98S	2 (18.1)	2 (22.2)	
M41L	1 (9)		1 (11.1)
M184V	1 (9)		1 (11.1)
L210W	1 (9)		1 (11.1)
T215Y	1 (9)		1 (11.1)
V108l	1 (9)		1 (11.1)
Y181C	1 (9)		1 (11.1)
H221Y	1 (9)		1 (11.1)

Table 3. Drug resistance mutations in protease inhibitor among HIV-1 patients.

Mutation	Frequency (%)		
	All (n = 11)	CRF01_AE (n = 9)	B (n = 2)
I84V	1 (9)	1 (11.1)	
L10l	4 (36.3)	3 (33.3)	1 (50)
G16E	2 (18.1)	2 (22.2)	
M36l	5 (45.4)	5 (55.5)	
H69K	4 (36.3)	4 (44.4)	
L89M	2 (18.1)	2 (22.2)	
L64V	1 (9)		1 (50)
V771l	1 (9)		1 (50)
I93L	3 (27.2)	2 (22.2)	1 (50)
K20R	2 (18.1)	2 (22.2)	

Table 4. Demographic characteristics of HIV-1 infected individuals with drug resistance to RT inhibitors and protease inhibitors.

ID	Subtype	Drug resistance mutation		
		RTI	PI	Resistance
RS-1	CRF01_AE	**K103N**[a]**, G190A**	**I84V**, L10l, G16E, M36l, H69K	ABC, **EFV**[b], ETR, **NVP**, RPV, **ATV**, DRV, **LPV**
RS-3	CRF01_AE	A98S	L10l, M36l, H69K, L89M	
RS-4		A98S		
RS-7	B	**M41L, M184V**, L210W, T215Y, V108l, Y181C, H221Y	L10l, l64V, V77l, I93L	ABC, AZT. FTC, 3TC, TDF, EFV, ETR, **NVP, RPV**
RS-10	CRF01_AE		L10l, G16E, K20R, M36l, H69K, L89M	
RS-11	CRF01_AE		M36l, H69K, I93L	
RS-12	CRF01_AE		K20R, M36l, I93L	

[a]Major drug resistance mutations are shown in bold.
[b]Highly resistant drugs are shown in bold.

4. Discussion

From total 11 samples which were analyzed, there were two samples with drug resistance-associated mutations, while the remaining had mutations but not yet shown any drug resistance. Samples with RS-1 ID have major mutations in reverse transcriptase genes in K103N and G190A where mutations in these genes cause resistance to Efavirenz (EFV) and Nevirapine (NVP). There is also amajor mutation in the protease genes I84V, causing resistance in all classes of protease inhibitors. Protease inhibitors are used as second-line therapy in HIV/AIDS management protocol in Indonesia. The sample has never been treated with a protease inhibitor before, so the mutations possibly happen due to transmitted drug resistance mutations. The sample is also mutated in the L101 gene causing mutations in virtually all protease inhibitors except Darunavir, a mutation in the G16E gene causing resistance to Atazanavir and M36I caused resistance to Atazanavir, Indinavir, Nelfinavir, and Tipranavir. Mutation in the H69K gene cause resistance to Tipranavir. So in the sample despite the resistance in all classes of protease inhibitors but still possible to receive treatment with darunavir because of low resistance. Although samples that infected by HIV-1 are mutated in all non-nucleoside reverse transcriptase inhibitor groups (NNRTIs) but still susceptible to most nucleoside reverse

transcriptase inhibitors (NRTIs) such as zidovudine (AZT), emtricitabine (FTC), lamivudine (3TC) and tenofovir (TDF).[8]

While in the sample with RS-7 ID there were a mutation in the reverse transcriptase (RT) genes in Thymidine Analogue-Associated Mutations, i.e.,on M41L, L120W, T215Y, thus causing resistance to all NRTIs except emtricitabine and lamivudine. But on the other hand also experienced a major mutation in M184V, so it causes resistance to emtricitabine, lamivudine, and abacavir. Mutations in the V108I and Y181C genes lead to resistance of NNRTIs group of nevirapine and efavirenz, whereas mutations in H221Y caused resistance to rilpivirine. In the protease genes are also reported minor mutations especially in L10, I64V, V77I, and I93L but not to cause resistance to antiretroviral therapy.[8] This sample is an injecting drug user (IDU) so that it is not only infected by HIV but also hepatitis C virus (HCV). This subject has been taking ART for 51 months with AZT, 3TC, and NVP ART regimens, but the subject had low medication adherence, which probably causes the occurrence of drug resistance-associated mutations. Patients phase of life, drug-related factors, and clinical stages arefactors that associated with sample adherence.[11]

HIV / AIDS patients are often hospitalized because of secondary infections. In patients who have received antiretroviral therapy, the reason for undergoing hospitalization becomes a question. Of the 11 samples that could be analyzed, two samples had drug resistance-associated mutations that affected the effectiveness of ART. While the other sample did not show mutation and showed susceptibility to the antiretroviral drugs being consumed, this is probably because some of the new samples received ART for less than one month. While others have some comorbid that can also affect the progression of the disease. Other factors, including adherence, previous hospitalization, pneumonia and injection drug use remained predictive of hospitalization.[12,13]

A large number of mutated samples pose a challenge in long-term antiretroviral treatment, so a breakthrough policy is needed to minimize the impact.

References

[1] Pustil R 2016 Global AIDS *Aids* **17(4)** S3-11 Available from: http://pesquisa.bvsalud.org/portal/resource/pt/mdl-15080170

[2] Didik B, Hardhana B, Yudianto M, Titi S, *et al*. 2017 *Profil kesehatan indonesia 2016* 100 Available from: http://www.depkes.go.id/resources/download/pusdatin/lain-lain/Data dan Informasi Kesehatan Profil Kesehatan Indonesia 2016 - smaller size - web.pdf

[3] Sungkanuparph S, Oyomopito R, Sirivichayakul S, Sirisanthana T, Li P C K, Kantipong P, *et al*. 2011 HIV-1 drug resistance mutations among antiretroviral-naive HIV-1-infected patients in Asia: results from the TREAT asia studies to evaluate resistance-monitoring study *Clin. Infect. Dis*. **52(8)** 1053–7

[4] Sungkanuparph S, Sukasem C, Kiertiburanakul S, Pasomsub E, Chantratita W 2012 Emergence of HIV-1 drug resistance mutations among antiretroviral-naive HIV-1-infected patients after rapid scaling up of antiretroviral therapy in Thailand *J. Int. AIDS Soc*. **15(1)** 12

[5] Kotaki T, Khairunisa S Q, Witaningrum A M, Yunifiar M M Q, Sukartiningrum S D, Diansyah M N, *et al*. 2015 HIV-1 transmitted drug resistance mutations among antiretroviral therapy-Naïve individuals in Surabaya, Indonesia *AIDS Res. Ther.* **12(1)** 5 Available from: http://aidsrestherapy.biomedcentral.com/articles/10.1186/s12981-015-0046-y

[6] Kotaki T, Khairunisa S Q, Sukartiningrum S D, Witaningrum A M, Rusli M, Diansyah M N, *et al*. 2014 Detection of drug resistance-associated mutations in human immunodeficiency virus type 1 integrase derived from drug-naive individuals in Surabaya, Indonesia *AIDS Res. Hum. Retroviruses* **30(5)** 489–92 Available from: http://online.liebertpub.com/doi/abs/10.1089/aid.2013.0271#utm_source=ETOC&utm_medium=email&utm_campaign=aid

[7] Khairunisa S Q, Kotaki T, Witaningrum A M, Yunifiar M M Q, Sukartiningrum S D, Nasronudin, *et al*. 2015 Appearance of drug resistance-associated mutations in human immunodeficiency virus type 1 protease and reverse transcriptase derived from drug-treated

Indonesian patients *AIDS Res. Hum. Retroviruses* **31(2)** 255–9
[8] Richman D 2013 *Update of the drug resistance mutations in HIV-1* 6–14
[9] Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods *Mol. Biol. Evol*. **28(10)** 2731–9
[10] Kimura M 1980 A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences *J. Mol. Evol*. **16(2)** 111–20
[11] Berheto T M, Haile D B and Mohammed S 2014 Predictors of loss to follow-up in patients living with HIV/AIDS after initiation of antiretroviral therapy *N. Am. J. Med. Sci*. **6(9)** 453–9
[12] Fielden S J, Rusch M L A, Levy A R, Yip B, Wood E, Harrigan R P, *et al* 2008 Predicting hospitalization among HIV-infected antiretroviral naïve patients starting HAART: determining clinical markers and exploring social pathways *AIDS Care* **20(3)** 297–303 Available from: http://dx.doi.org/10.1080/09540120701561296
[13] Palepu A, Sun H, Kuyper L, Schechter M T, O'Shaughnessy M V and Anis A H 2003 Predictors of early hospital readmission in HIV-infected patients with pneumonia *J. Gen. Intern. Med*. **18(4)** 242–7