Genotypic Analysis of Transmitted and Acquired HIV Drug Resistance in People Living with HIV/AIDS in Surabaya, Indonesia, from 2018 to 2019

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ABSTRACT

Background: *Despite the availability of various effective antiretroviral (ARV) drugs, human immunodeficiency virus (HIV) infection has come with HIV drug resistance (HIVDR), which compromises its effectiveness in reducing HIV-related morbidity, mortality, and transmission. The emergence of transmitted (TDR) and acquired HIVDR (ADR) among antiretroviral therapy (ART)-naïve and experienced individuals have been reported in several Indonesian regions. Therefore, continuous HIVDR surveillance is needed in Indonesia, especially in Surabaya, which is identified as having the highest prevalence of HIV infection in East Java; thus, this study aimed to identify the emergence of TDR and ADR among people living with HIV/acquired immune deficiency syndrome (AIDS) (PLWHA).* ***Methods:*** *Fifty-eight PLWHA infected with HIV type 1 (HIV-1), comprising 21 and 37 ART-naïve and experienced individuals were enrolled in this study, respectively. Blood samples collected from study participants were subjected to genotypic analysis, mainly towards the pol gene encoding protease (PR gene) and reverse transcriptase (RT gene) of HIV-1.* ***Results:*** *Seventeen PR and 21 RT genes were successfully amplified and sequenced from 29 samples. HIV-1 subtyping revealed CRF01_AE as the most dominant subtype (24/29; 82.76%), followed by subtype B (3/29; 10.34%). Uncommon subtypes, including subtype D and a recombinant containing subtypes B and G genomic fragments, were also identified. TDR for PR inhibitors was not detected; however, TDR and ADR for RT inhibitors were identified in 11.11% and 41.67% of samples, respectively. Two amino acid insertions at position 69 of the RT gene (69ins), a previously never-reported mutation in Indonesia, were identified in this study.* ***Conclusion:*** *Both TDR and ADR have emerged among PLWHA residing in Surabaya,*

East Java, Indonesia. Uncommon drug-resistance mutations and subtypes were identified in this study. These situations might hamper ART efficacy and treatment success. Continuous surveillance of HIVDR is necessary to monitor both TDR and ADR in Indonesia.

Keywords: *HIV-1, Surabaya, antiretroviral therapy (ART), transmitted HIV drug resistance (TDR), acquired HIV drug resistance (ADR).*

INTRODUCTION

The human immunodeficiency virus (HIV) infection remains a major public health problem, claiming approximately 650,000 lives in 2021. Despite the existing efforts to reduce its transmission, more than one million new HIV infections have been reported annually since its peak in 1996. About 38.4 million people in the world were living with HIV/-acquired immune deficiency syndrome (AIDS) (PLWHA), including 15% of those who did not know their HIV status.[1]

The World Health Organization (WHO) recommends all individuals infected with HIV receive antiretroviral therapy (ART), which typically includes three medications from two antiretroviral (ARV) drug classes. Currently, the Food and Drug Administration (FDA) of the United States of America has approved nine classes of HIV medication, including nucleoside reverse transcriptase inhibitors (nRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), attachment inhibitors, CCR5 antagonists, fusion inhibitor, post-attachment inhibitors, and pharmacokinetic enhancers.[2] Before September 2022, the Indonesian Ministry of Health recommended two drugs from the nRTI class and one drug from the NNRTI class as the first-line ART regimen; thus, PLWHA in Indonesia relied heavily on nRTI and NNRTI-based regimens. Two NRTIs plus one boosted-PI, and one boosted-PI, one INSTI, and one-to-two nRTI were recommended as second and third-line regimens, respectively.[3]

ART was not only effective in achieving viral suppression and CD4 cell count recovery but also in improving physical, and psychological, levels of independence, environment, and spiritual quality of life.[4–6] However, despite the availability of various effective ARV drugs, the management of HIV infection now faces the emergence of HIV drug resistance (HIVDR), which compromises effectiveness in reducing HIV-related morbidity, mortality, and transmission. HIVDR towards NNRTIs can affect more than 10% of ART-naive adult individuals and is found up to three times more often among ART-experienced individuals.[7,8]

Genotypic studies in Indonesia have identified several major transmitted HIVDR (TDR) among ART-naive and acquired HIVDR (ADR) among ART-experienced PLWHA residing in several Indonesian regions.[9–15] Two studies published in 2015 reported the prevalence of TDR and ADR was 4.3% and 37.7% in Surabaya, respectively.[16,17] These situations suggest the necessity of continuous HIVDR surveillance in Indonesia, especially in Surabaya, with the highest prevalence of HIV type 1 (HIV-1) infection in East Java. In 2019, the prevalence of HIV-1 infection in East Java was the fourth highest among Indonesian provinces.[18] This study aimed to identify the emergence of TDR and ADR among PLWHA in 2018-2019 through genotypic analysis.

METHODS

This study was approved by the Institutional Ethics Committees of Universitas Airlangga (Approval No.: 25-995/UN3.14/PPd/2013) and Kobe University Graduate School of Medicine (Approval No.: 784). The inclusion criteria of research participants were those: 1) at 18 years and older; 2) already diagnosed with HIV-1 infection by an authorized healthcare provider; and 3) giving written informed consent to enroll in the study.

Sample collection was performed at a Teaching Hospital in Surabaya from late 2018 to early 2019. Five milliliters of ethylenediaminetetraacetic acid (EDTA)-

anticoagulated peripheral blood samples were collected from each participant. Cellular DNA was extracted from whole blood using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany), followed by PCR amplification and sequencing analysis of viral *pol* gene encoding reverse transcriptase (RT gene) and protease (PR gene), as described in Khairunisa et al.[9] New sequence data and corresponding viral gene fragments of reference HIV-1 strains retrieved from the HIV sequence database (www.hiv.lanl.gov/) were compiled and aligned using MEGA7 software.[19]

A phylogenetic tree analysis performed HIV-1 subtyping, jumping profile Hidden Markov Model (jpHMM)-HIV tools (http://jphmm.gobics.de/submission_hiv), and recombinant identification program (RIP) (www.hiv.lanl.gov/). Neighbor-joining (NJ) trees with the Kimura two-parameter model were constructed using MEGA7 software,[19] with bootstrap values (1,000 replicates) for relevant nodes reported on a representative tree. Identification of drug resistance mutations (DRMs) in successfully sequenced PR and RT genes was based on the International Antiviral Society-United States (IAS-USA) guidelines.[20] Transmitted and acquired HIVDR were then defined as the presence of at least one major DRM in sequences obtained from ART-naïve and experienced individuals, respectively. The level of resistance towards ARV was determined based on the HIVDR database of Stanford University (https://hivdb.stanford.edu/hivdb/by-patterns/). The nucleotide sequences of viral gene fragments have been deposited in the GenBank database under accession numbers OR547957-OR547994.

RESULTS

Participants Characteristics

Fifty-eight PLWHA, comprising of 21 ART-naïve and 37 ART-experienced individuals, were enrolled in this study. Overall, the predominant age group was 18-30 years old (20/58; 34.48%). Most participants were male (43/58; 74.14%) and acquired HIV-1 infection through a heterosexual transmission route (44/58; 68.97%). Among ART-experienced individuals, 91.89% (34/37) received first-line ART regimen, comprising of lamivudine (3TC) + zidovudine (AZT) + nevirapine (NVP) (21/37; 56.76%), 3TC + AZT + efavirenz (EFV) (1/37; 2.7%), 3TC + TDF + NVP (1/37; 2.7%), and 3TC + tenofovir (TDF) + EFV (11/37; 29.73%). Three individuals (3/37; 8.11%) received a second-line ART regimen containing 3TC + TDF + ritonavir-boosted lopinavir (LPV/r). Thirty-seven (63.79%) participants were Javanese, while the rest were Chinese and of other ethnicities. The characteristics of the participants are shown in **Table 1**.

Table 1. Demographic Characteristics of Study Participants.

	ART-naïve (n)	%	ART-experienced (n)	%	Total (n)	%
Age						
18-30	11	32.35	9	24.32	20	34.48
31-40	5	14.71	10	27.03	15	25.86
41-50	4	11.76	12	32.43	16	27.59
51-60			3	8.11	3	5.17
>60	1	2.94	3	8.11	4	6.90
Sex						
Male	17	80.95	26	70.27	43	74.14
Female	4	19.05	11	29.73	15	25.86
Transmission						
Heterosexual	18	85.71	22	59.46	40	68.97
Homosexual	3	14.29	7	18.92	10	17.24
IDU			8	21.62	8	13.79
ART Regimen						
3TC (L) + TDF + LPV/r	N/A	-	3	8.11		
3TC+AZT+NVP	N/A	-	21	56.76		
3TC-TDF+EFV	N/A	-	11	29.73		

3TC+TDF+NVP	N/A	-	1	2.70		
3TC+AZT+EFV	N/A	-	1	2.70		
Ethnicity						
Javanese	18	85.71	19	51.35	37	63.79
Chinese	2	9.52	13	35.14	15	25.86
Other	1	4.76	5	13.51	6	10.34

3TC – lamivudine; AZT – zidovudine; TDF – tenofovir; NVP – nevirapine; EFV – efavirenz; LPV/r – ritonavir-boosted lopinavir

HIV-1 Subtype

Sequencing data of 17 PR genes [297 base pairs (bp); nt 2253–2549 by HXB2 numbering, and 21 N-terminus of RT genes (741 bp; nt 2571–3311) were successfully sequenced from cellular DNA extracted from 29 PLWHA. NJ trees for the PR and RT genes are shown in **Figure 1**. Viral subtyping by RIP, jpHMM-HIV, and phylogenetic trees showed consistent results (data not shown). Twenty-four samples (24/29; 82.76%) were classified as CRF01_AE, three samples (3/29; 10.34%) were subtype B, one sample (1/29; 3.45%) was subtype D, and one sample (1/29; 3.45) was a recombinant virus containing subtypes B and G genomic fragments (B/G recombinant).

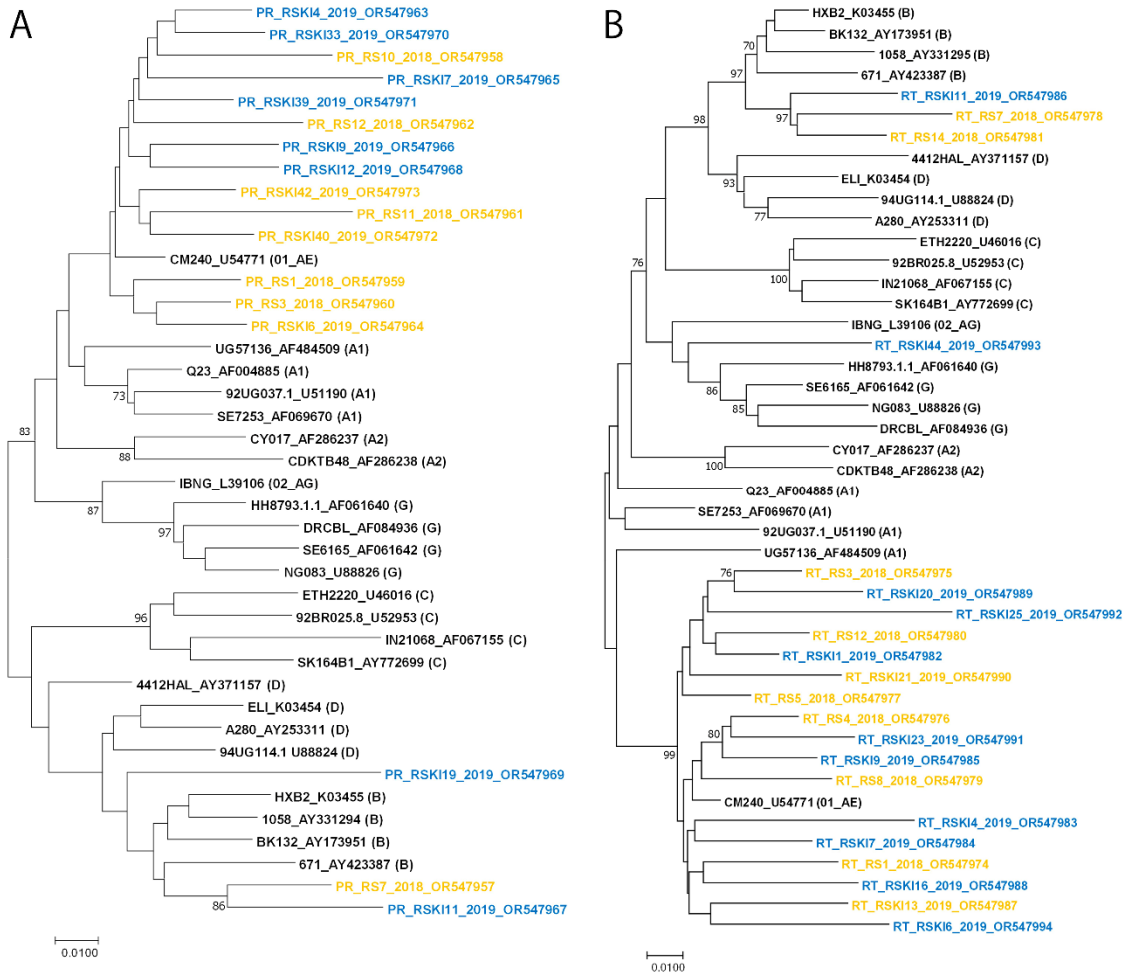

Figure. 1. Phylogenetic tree analysis of HIV-1 PR and RT gene sequences collected from ART-naïve and experienced individuals residing in Surabaya, East Java, Indonesia. Blue and orange colors denote samples from ART-naïve and experienced individuals, respectively.

Phylogenetic trees were constructed for the HIV-1 PR (A) and RT (B) genes newly sequenced in the present study. The corresponding viral genes of reference HIV-1 strains representing subtypes A1, A2, B, C, D, and G, as well as CRF01_AE (01_AE) and CRF02_AG (02_AG) were included in the analyses (shown in black color). Sequence IDs are presented as a sample ID or the ID of the reference HIV-1 strain, a GenBank accession number, and the subtype or CRF of the reference strain (shown in parentheses) in that order. Bootstrap values were shown if they were >70.

Detection of TDR

Eight PR and 9 RT genes were successfully sequenced from 14 ART-naïve individuals. TDR related to the PR genes was not observed; however, several minor mutations were identified, including L10I/V [amino acid substitution from leucine (L) to isoleucine (I) or valine (V) at position 10 in the PR gene] (2/8; 25%), K20R (3/8; 37.5%), M36I (6/8; 75%), I62V (2/8; 25%), H69K (6/8; 75%), A71T (1/8; 12.5%), V77I (3/8; 37.5%), and L89M (6/8; 75%). One sample (1/9; 11.11%) possessed TDR, the G190A mutation, in the RT genes (as presented in **Table 2**).

Detection of ADR

Nine PR and 12 RT genes were successfully sequenced from 14 ART-experienced individuals. Similar to those of ART-naïve individuals, there was also no ADR in the PR genes of ART-experienced individuals. Several minor mutations in the PR genes were identified, including L10I/F (5/9; 55.55%), K20R (2/9; 22.22%), L33F (1/9; 11.11%), M36I (8/9; 88.89%), H69K (8/9; 88.89%), V77I (1/9; 11.11%), and L89M (7/9; 77.78%). Five samples (5/12; 41.67%) possessed ADR in the RT genes. The most common major RT genes mutation observed was M184V/I (4/12; 19.05%), followed by M41L (3/12; 14.29%), T215Y/F (3/12; 14.29%), K101P/E (2/12; 9.52%), K103N/S (2/12; 9.52%), Y181C (2/12; 9.52%), and G190A (2/12; 9.52%). Other mutations, including A62V, D67N, 69ins (S-A) [2-amino acid (serine and alanine) insertion in the following position 69 in the RT gene], K70R, V108I, L210W, and H221Y, were each observed in one sample (1/12; 4.76%). Individual samples with major DRMs in RT genes and levels of resistance toward ARV are shown in **Table 2**.

Table 2. HIV-1 Drug Resistance Mutations of Individual Samples

Sample Code	ART status	Subtype	nRTI-related mutations	NNRTI-related mutations	Levels of ARV resistance
RS1_2018	Experienced	CRF01_AE		K103N, G190A	High-level resistance to EFV and NVP, low-level resistance to RVP, and potential low-level resistance to ETV.
RS7_2018	Experienced	B	M41L, M184V, L210W, T215Y	V108I, Y181C, H221Y	High-level resistance to ABC, AZT, FTC, 3TC, NVP, and RPV. Intermediate resistance to TDF, DOR, EFV, and ETV.
RSKI6_2019	Experienced	CRF01_AE	M41L, A62V, 69INS, M184V, T215Y	K101E, Y181C, G190A	High-level resistance to AZT, FTC, 3TC, NVP, EFV, ETV, and RPV. Intermediate resistance to ABC, and DOR, and low-level resistance to TDF.
RSKI13_2019	Experienced	CRF01_AE	M41L, D67N, K70R, M184V, T215F	K101P, K103S	High-level resistance to ABC, AZT, FTC, 3TC, EFV, ETV, NVP, and RPV. Intermediate resistance to TDF, and potential low-level resistance to DOR
RSKI21_2019	Experienced	CRF01_AE	M184I		High-level resistance to FTC and 3TC, and low-level resistance to ABC.
RSKI25_2019	Naïve	CRF01_AE		G190A	High-level resistance to NVP, intermediate resistance to EFV, low-level resistance to RPV, and potential low-level resistance to ETV.

ABC – Abacavir, FTC – Emtricitabine, 3TC – Lamivudine, TDF – Tenofovir, AZT – Zidovudine, ddI – Didanosine, d4T – Stavudine, DOR – Doravirine, EFV – Efavirenz, ETV – Etravirine, NVP – Nevirapine, RPV – Rilpivirine

DISCUSSION

We herein report the circulating HIV-1 subtype and prevalence of HIVDR among HIV-1-infected, ART-naïve, and experienced individuals in Surabaya, East Java, Indonesia. Among 28 successfully sequenced samples, the most common HIV-1 subtype identified was the CRF01_AE (24/29; 82.76%). This finding is consistent with those previously reported in Surabaya,[17,21], and with the reports in several other Indonesian regions, including Bali, Jakarta, Pontianak, and Makassar.[9–14] Subtype B was identified in three samples (3/29; 10.34%) Along with CRF02_AG, subtype B was the second most-identified HIV-1 subtype in Indonesia.[9,10,12] Other subtypes, including subtype D and the B/G recombinant identified in this study, have not previously emerged in Indonesia. Subtype D was mostly found in East and Central Africa,[22] while B/G recombinant was previously reported in Mexico, Spain, and Portugal.[23,24] However, it is necessary to carry out further analysis in a future study, using other gene fragments or carrying out nearly full-length genomic sequencing to determine whether unique HIV-1 subtypes and recombinants were circulating in Indonesia. The HIV-1 gene fragments analyzed in the present study were considered insufficient to identify actual recombinant forms.

Compared to HIV genotypic studies conducted in Surabaya in 2015,[16,17] TDR and ADR in the present study showed a higher prevalence of 11.11% (1/9) and 41.67% (5/12), respectively. Previously, prevalence rates of 4.3% and 37.7% for TDR and ADR were reported in Surabaya, respectively.[16,17]

Several major DRMs in the RT genes were identified in ART-experienced individuals, including M41L, D67N, K101P/E, K103N/S, V108I, Y181C, M184V/I, L210W, T215Y/F, and H221Y. These mutations were associated with drug resistance to AZT, stavudine (d4T), abacavir (ABC), EFV, etravirine (ETR), NVP, and rilpivirine (RPV). A mutation, A62V is related to 151 complex that affects all nRTIs currently approved by the FDA, excluding TDF. In addition, mutations, K70R, L210W, and T215Y/F are also related to Thymidine Analogue-Associated Mutations or TAMs, which affect all approved nRTIs other than emtricitabine (FTC) and 3TC.[20] Amino acid insertion at amino acid position 69, along with A62V, K70R, and L210W, T215Y/F are related to the 69-insertion complex that affects all nRTIs currently approved by the FDA. Interestingly, the 69ins mutation described in this study has not been reported in previous genotypic studies carried out in Indonesia. A mutation, G190A was identified in both ART-naïve and -experienced individuals. This mutation affects EFV and NVP,[20] two NNRTIs that were mainly used in first-line ART regimens.[3]

This current study revealed no evidence of circulating PI-related TDR and ADR in Surabaya. This may have been due to the limited usage of PIs. Among ART-experienced individuals enrolled in this study, only 8.11% received a second-line ART regimen containing PI drug class. The Indonesian Ministry of Health reported that 123,895 PLWHA (97%) received first-line ART regimens and 3,718 individuals (3%) received second-line ART regimens in 2019.[18]

Despite no major DRMs being detected in the PR genes, minor mutations, including L10I/F/V, K20R, L33F, M36I, I62V, H69K, A71T, V77I, and L89M, were identified in both ART naïve and experienced PLWHA enrolled in this study. These mutations might potentially affect viral susceptibility to ritonavir-boosted atazanavir (ATV/r), ritonavir-boosted lopinavir (LPV/r), ritonavir-boosted tipranavir (TPV/r), ritonavir-boosted fosamprenavir (FPV/r), ritonavir-boosted indinavir (IDV/r), nelfinavir (NFV), and ritonavir-boosted saquinavir (SQV/r).[20] Previous studies confirmed the presence of similar PI-related minor DRMs among PLWHA in other Indonesian regions, including Bali, Jakarta, Pontianak, and Makassar.[9–14] The presence of these minor mutations, especially those affecting the efficacy of LVP/r, might need to be considered when a second-line regimen is being recommended for PLWHA in Indonesia.[3]

The results of the present study should raise awareness of TDR and ADR present in PLWHA residing in Surabaya, East Java, Indonesia. According to the WHO, the prevalence of HIVDR in a geographical area is categorized

into three groups: low level (<5%), moderate level (5–15%), and high level (>15%).[25] Based on these categories, the prevalence of TDR and ADR in Surabaya in 2018-2019 was considered to be moderate and high, respectively. However, current results may have overestimated the prevalence due to the limitation in the study design. The number of samples collected was limited; therefore, continuous monitoring of TDR and ADR with a larger sample size is necessary.

CONCLUSION

Both TDR and ADR emerged in PLWHA residing in Surabaya, East Java, Indonesia. A drug resistance mutation that was previously not reported, 69ins, along with uncommon HIV-1 subtypes (subtype D and B/G recombinant), have been identified in this study. These situations warrant serious consideration due to the hampering impact of drug resistance on ART efficacy and treatment success. Continuous surveillance of HIVDR, especially with a larger sample size, is necessary to monitor TDR and ADR in Indonesia. Besides, it is necessary to strengthen the national policy regarding the appropriate choice of ART.

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COMPETING INTEREST

The authors declared no competing interest.

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