The Dominance of CRF01_AE and the Emergence of Drug Resistance Mutations Among Antiretroviral Therapy-Experienced, HIV-1-infected Individuals in Medan, Indonesia

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ABSTRAK

Latar belakang: *infeksi human immunodeficiency virus tipe 1 (HIV-1) adalah ancaman kesehatan masyarakat yang serius di seluruh dunia. Medan merupakan salah satu kota besar di Indonesia dengan prevalensi infeksi HIV-1 yang tinggi; namun, penelitian yang terbatas untuk mendeteksi peredaran subtipe HIV-1 di Medan. Selain itu, faktor serius yang dapat berimplikasi pada pengobatan orang yang terinfeksi HIV-1 adalah munculnya mutasi yang resisten terhadap obat. Oleh karena itu, informasi tentang infeksi HIV-1 penting untuk meningkatkan pengobatan bagi individu yang terinfeksi.* ***Metode:*** *enam puluh tujuh orang yang berpengalaman dengan terapi antiretroviral, orang yang terinfeksi HIV-1 diikutsertakan pada penelitian ini. Gen HIV-1 pol yang mengkode protease (gen PR) dan reverse transcriptase (gen RT), serta gen env dan gag, diamplifikasi dari DNA yang berasal dari sampel darah tepi. Subtipe HIV-1 dilakukan untuk mempelajari subtipe HIV-1 dominan yang beredar di wilayah tersebut. Selain itu, munculnya mutasi yang resisten terhadap obat dianalisis berdasarkan pedoman yang diterbitkan oleh International Antiviral Society-United States of America (IAS-USA).* ***Hasil:*** *subtipe HIV-1 yang dominan ditemukan di Medan adalah CRF01_AE (77,6%). Selain itu, subtipe dan virus rekombinan lain seperti rekombinan pada CRF01_AE dan subtipe B (12,2%), subtipe B (4,1%), dan CRF02_AG (4,1%) juga ditemukan. Mutasi utama terkait resistensi obat ditemukan pada 21,6% (8/37) gen RT dan 3,1% (1/32) gen PR yang diteliti.* ***Kesimpulan:*** *penelitian ini menunjukkan bahwa subtipe dominan yang ditemukan pada orang yang berpengalaman dengan ART, orang yang terinfeksi HIV yang tinggal di Medan adalah CRF01_AE. Munculnya mutasi yang resisten terhadap obat dalam gen RT dan PR menunjukkan pentingnya memantau prevalensi mutasi yang resisten terhadap obat di antara orang yang terinfeksi HIV-1 di Medan.*

Kata kunci: *HIV-1, CRF01_AE, terapi antiretroviral, resistensi obat, Medan.*

ABSTRACT

Background: *human immunodeficiency virus type 1 (HIV-1) infection is a serious public health threat worldwide. Medan is one example of big cities in Indonesia with a high prevalence of HIV-1 infection; however, quite a limited study had conducted for detecting the circulation of HIV-1 subtypes in Medan. In addition, a serious*

factor that can implicate the treatment of HIV-1-infected individuals is the emergence of drug resistance mutations. Thus, the information on HIV-1 infection is important to improve the treatment for infected individuals. ***Methods:*** *sixty-seven antiretroviral therapy-experienced, HIV-1-infected individuals were recruited for this study. HIV-1 pol genes encoding protease (PR genes) and reverse transcriptase (RT gene), as well as env and gag genes, were amplified from DNA derived from peripheral blood samples. HIV-1 subtyping was conducted to study the dominant HIV-1 subtype circulating in the region. In addition, the emergence of drug resistance mutations was analyzed based on the guidelines published by the International Antiviral Society-United States of America (IAS-USA).* ***Results:*** *the dominant HIV-1 subtype found in Medan was CRF01_AE (77.6%). In addition, another subtype and recombinant viruses such as recombinants between CRF01_AE and subtype B (12.2%), subtype B (4.1%), and CRF02_AG (4.1%) were also found. Drug resistance-associated major mutations were found in 21.6% (8/37) of RT genes and 3.1% (1/32) of PR genes studied.* ***Conclusion:*** *our study showed that the dominant subtype found in ART-experienced, HIV-1-infected individuals residing in Medan was CRF01_AE. The emergence of drug resistance mutations in RT and PR genes indicated the importance to monitor the prevalence of drug resistance mutations among HIV-1-infected individuals in Medan.*

Keywords: *HIV-1, CRF01_AE, antiretroviral therapy, drug resistance, Medan.*

INTRODUCTION

The number of reported HIV-1-infected cases in Indonesia has increased in the last decade, 2010-2018 with the highest number reported in 2016-2018. The data from the Ministry of Health showed that, in 2017, there were 48,300 HIV-1 infection cases. Meanwhile in 2018, the number dropped to 46.659 cases. In North Sumatera, the number of reported HIV-1 infection in 2017 and 2018 was 1,891 and 1,999 cases, respectively, and it was ranked seventh in the number of HIV-1-infected individuals among provinces in Indonesia.[1] Since Medan is the capital city of North Sumatera province, it is important to study the HIV-1 infection in this city.

HIV-1 infection was successfully suppressed by the antiretroviral therapy (ART). Recommended first-line regimens by WHO in 2019 are dolutegravir (DTG) in combination with a nucleoside reverse transcriptase (RT) inhibitor (NRTI) backbone, and efavirenz (EFV) in combination with an NRTI backbone as the alternative first-line regimen for HIV-infected adult. For infants and children, WHO recommends raltegravir (RAL)-based regimen.[2] The first-line ART regimens recommended by the Indonesian Ministry of Health are tenofovir (TDF), lamivudine (3TC) or emtricitabine (FTC) with efavirenz (EFV). Alternative first-line regimens which can be applied for HIV-1 infected individuals are 1). Zidovudine (AZT), 3TC with EFV or nevirapine (NVP); and 2). TDF, 3TC or FTC with NVP.

Recently, the prevalence of drug resistance has increased among individuals on ART in Indonesia.[3,4] This drug resistance can decrease the efficacy of ART. Meanwhile, it is known that there are two types of drug resistance which acquired drug resistance and transmitted drug resistance (TDR), and TDR is believed to have greater impact on ART.[5] The prevalence of drug resistance mutations was also found in Surabaya and Maumere, Indonesia, in our previous studies.[6,7] Thus, it is important to monitor the effectiveness of ART and prevalence of drug resistance mutations in other cities in Indonesia.

We aimed to monitor the prevalence of drug resistance mutations among HIV-1-infected individuals on ART. In addition, to reveal the prevalence of drug resistance mutations, molecular epidemiological study to determine the dominant HIV-1 subtype is also needed. A dominant HIV-1 subtype in Indonesia is CRF01_AE[8]; however, it cannot rule out the emergence of other subtypes in Medan since the information on prevalent HIV-1 subtype is quite scarce in this region.

METHODS

Sixty seven HIV-1-infected individuals on ART (33 male and 34 female, the mean age of 31 years old) were recruited from VCT

program in community health centers in Padang Bulan Medan, North Sumatera, Indonesia. Ten milliliters of ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood samples were collected from participants using BD vacutainer®CPT (BD Bioscience, San Jose, USA) in July 2017. Peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation with histopaque (Sigma Aldrich, USA). After centrifugation at 2,000rpm for 20 minutes, blood samples were separated into plasma, PBMC and red blood cells. After the centrifugation, thin layer ring or buffy coat containing PBMC was formed. DNA was extracted from PBMC using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany).

This study was conducted with an approval from the Ethics committees of Universitas Airlangga and Kobe University Graduate School of Medicine. All study participants were requested to sign an informed consent prior to sample calculation. All participants agreed to provide blood samples for this study.

Polymerase Chain Reaction (PCR) and Sequence analysis

The partial fragments of viral pol gene encoding protease (PR gene) and RT (RT gene), as well as gag and env genes, were amplified from DNA extracted from PBMC samples by a nested PCR using Ex Taq (Takara Bio, Shiga, Japan) and the following primers. For the amplification of RT gene, the primers for the first PCR were RT1L, 5'-ATGATAGGGGGAATTGGAGGTTT-3' [corresponding to nucleotide (nt) 2388 to 2410 of a HIV-1 reference strain, HXB2 (GenBank accession no. K03455)] and GPR2M, 5'-GGACTACA GTCYACTTGTCCATG-3' (nt 4402 to 4380), and the primers for the nested PCR were RT7L, 5'-GACCTAC ACCTGTCAACATAATTGG-3' (nt 2485 to 2509) and GPR3L, 5'-TTAAAATCACTARCCATTGYTCTCC-3' (nt 4309 to 4285). For the amplification of PR gene, the primers for the first PCR were PRO5F, 5'-AGAAATTGCAGGGCCCCTAGGAA-3' (nt 2022 to 2044) and DRPR02L, 5'-TATGGATTTTCAGGCCCAATTTTTGA-3' (nt 2716 to 2691), and the primers for nested PCR were PRO5F and DRPR06, 5'-ACTTTTGGGCCATCCATTCC-3' (nt 2611 to 2592). The viral env gene was amplified by nested PCR with the primers M5, 5'-CCAATTCCCATACAT TATTGTGCCCCAGCTGG-3' (nt 6858 to 6889), and M10, 5'-CCAATTGTCCCT CATATCTCCTCCTCCAGG-3' (nt 7661 to 7632), in the first round, and M3,5'-GTCAGCACAGTACAATGIACACATGG-3' (nt 6948 to 6973), and M8, 5'-TCCTTCCATGGGA GGGGCATACATTGC-3' (nt 7547 to 7521), in the second round. The viral gag gene was amplified by nested PCR with the primers H1G777, 5'-TCACCTAGAACTTTGAATGCATGGG-3' (nt 1231 to 1255), and H1P202, 5'-CTAATACTGTATCATCTGCT GCTCCTGT-3' (nt 2352 to 2325), in the first round, and H1Gag1584, 5'-AAAGATGGATAATCCTGGG-3' (nt 1577 to 1595), and G17, 5'-TCCACATTTC CAACAGCCCTTTTT-3' (nt 2040 to 2017), in the second round. PCR products were then visualized by ethidium bromide staining under UV light following 1.5% agarose gel electrophoresis. All successfully amplified samples for PR, RT, gag and env genes were then subjected to sequencing analysis using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) with an ABI PRISM 3500 xl genetic analyzer following purification step using ExoSap-IT (ThermoFisher Scientific, Waltham, USA). Sequencing data were then assembled and aligned using Genetyx version 10 software (Genetyx, Tokyo, Japan). As the results, the sequencing data of 32 PR genes (297-bp), the N-terminus of 37 RT genes (762-bp), 34 env genes spanning C2-V3 region of gp120 (389-bp) and 28 gag genes encoding a part of Gag p24 (382-bp) were obtained from 67 blood samples. The nucleotide sequences of these PR, RT, gag and env genes have been deposited in the GenBank database under accession numbers MT163520-MT163650.

HIV-1 Subtyping and the Detection of Drug Resistance Mutations

HIV-1 subtyping was carried out using the recombinant identification program (RIP), available at the website of the HIV sequence database (www.hiv.lanl.gov/). In addition,

neighbor-joining (NJ) trees with a Kimura two-parameter model were constructed using MEGA6.2 software13 with Bootstrap values (1,000 replicates) as comparison with those results analyzed with RIP. Viral subtyping was carried out based on the successfully sequenced PR, RT, env and gag genes, and if there were an incompatibility in the subtype or CRF among the PR, RT, gag and env genes, the viral gene was considered to be a recombinant form of HIV-1.

Drug resistance-associated mutations were studied according to the International Antiviral Society-United States (IAS-USA) report.[9] We analyzed drug resistance-associated mutations on RT and PR genes which were the targets of the first line and the second line ART regimens in Indonesia, respectively.

RESULTS

Medan is one of the big cities in Indonesia. The majority of HIV-1-infected individuals were Batak and Javanese. HIV transmission in Medan was spread dominantly through sexual intercourse while most (82.8%) study participants were married (**Table 1**). All study participants had experienced ART more than a year. They were treated with the first line ART regimen as it is suggested for primary treatment by Ministry of Health in accordance with WHO regulations.

CRF01_AE was the Dominant HIV-1 Subtype Found in Medan

HIV-1 subtypes detected among HIV-1-infected individuals in Medan were CRF01_AE (41 samples), recombinants between CRF01_AE and subtype B (6 samples), CRF02_AG (2 samples), and subtype B (2 samples) (**Figure 1**). The result showed that the dominant HIV-1 subtype in Medan was CRF01_AE, indicating that the dominant subtype found in this region was similar to that in other regions in Indonesia. Since its first appearance in 1990s,10 CRF01_AE has been continuously found as a dominant subtype in Indonesia. CRF01_AE was found in 3 individuals with IDU, 4 wives with heterosexual transmission from husband with IDU, 17 individuals with heterosexual transmission, 13 homosexual individuals (men who have sex with men or MSM), 6 pediatric patients transmitted from mother, and an individual with blood transfusion.

Table 1. General Characteristic of HIV-1-infected Individuals in Medan.

Characteristics	Value (n=67)
Age, mean (SD), years	31.0 (10.8)
Gender, n (%)	
- Male	33 (49.3)
- Female	34 (50.8)
Marital Status, n (%)	
- Married	48 (82.8)
- Single (Divorced/Widowed)	10 (17.2)
Ethnic group, n (%)	
- Batak	26 (38.8)
- Malay	5 (7.5)
- Javanese	23 (34.3)
- Chinese	5 (7.5)
- Dayak	1 (1.5)
- Indian	1 (1.5)
- Karo	1 (1.5)
- Mandailing	1 (1.5)
- Minang	2 (3.0)
- Nias	2 (3.0)
Transmission risk category, n (%)	
- Heterosexual	34 (50.8)
- Injecting drug use (IDU)	7 (23.9)
- Men having sex with men	16 (23.9)
- Mother to Child Transmission (MTCT)	8 (12.0)
- Blood transfusion	2 (3.0)
Types of ART used, n (%)	
- AZT+3TC+NVP	24 (35.8)
- AZT+3TC+EFV	10 (14.9)
- TDF+3TC+EFV	21 (31.3)
- TDF+3TC+NVP	10 (14.9)
Duration of ART, n (%)	
- < 1 year	3 (4.5)
- 1 – 3 years	24 (35.8)
- > 3 years	40 (59.7)

Recombinants between CRF01_AE and subtype B as well as CRF02_AG were found among individuals with heterosexual transmission. In addition, subtype B was found in MSM and individuals with heterosexual transmission.

Major and Minor Drug Resistance Mutations Detected in RT Genes

Most study participants were on ART with first-line regimens, AZT, 3TC and NVP/EFV; or TDF, 3TC and NVP/EFV. Drug resistance mutations were detected among 9 out of 37

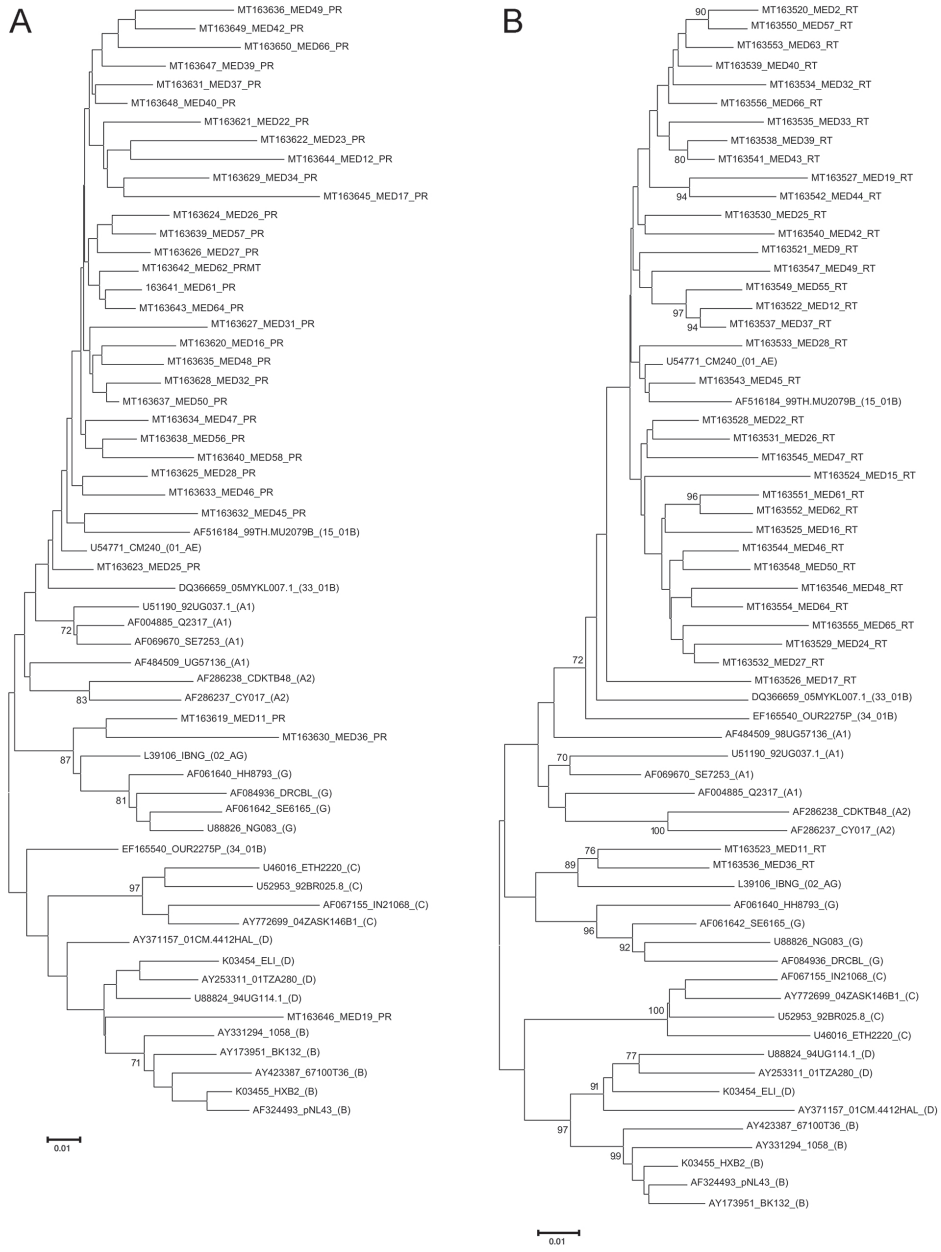
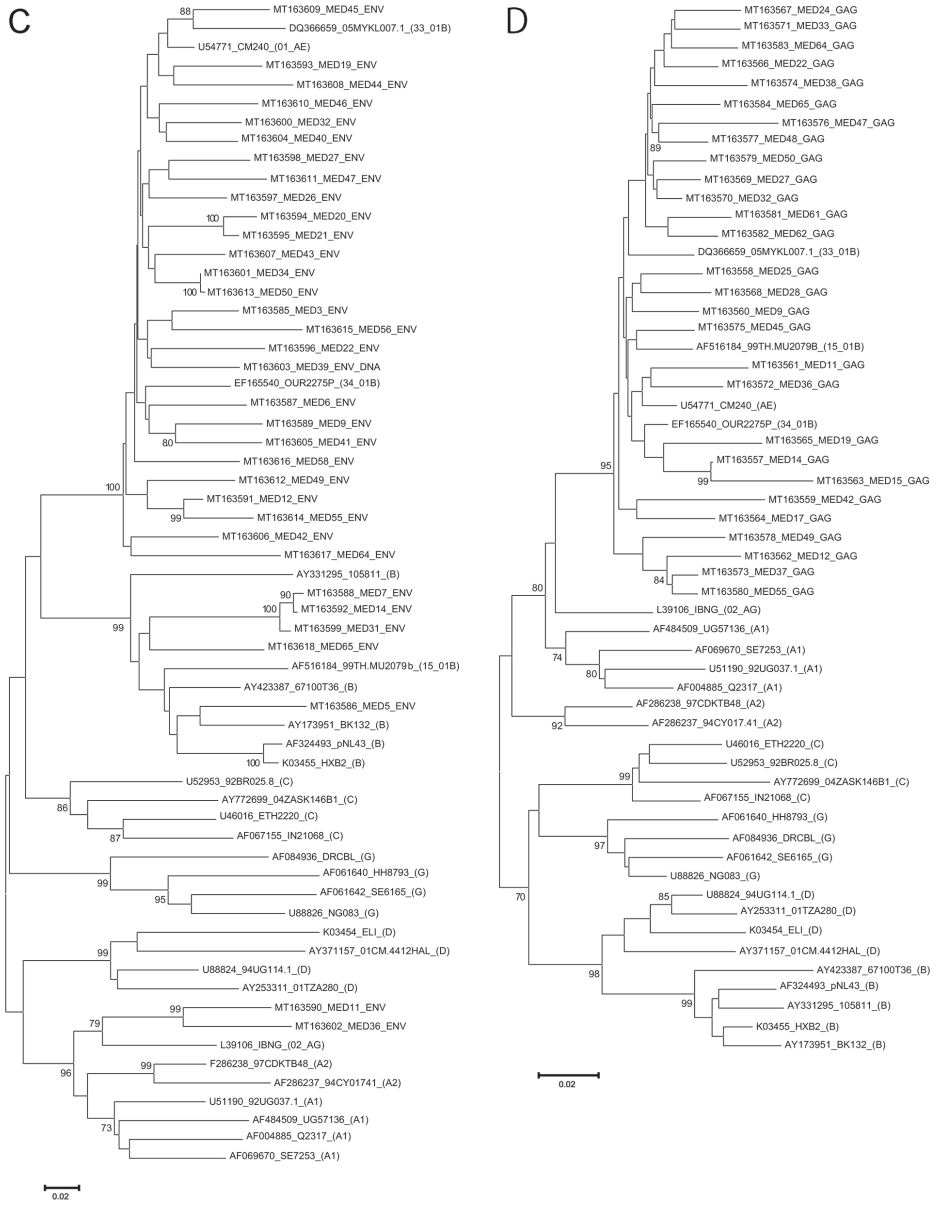


Figure 1. Phylogenetic tree analysis of HIV-1 RT, PR, env, and gag genes derived from infected individuals residing in Medan, Indonesia. Phylogenetic trees were constructed for the HIV-1 RT (A), PR (B), env (C), and gag genes newly sequenced in the present study (D). The corresponding viral genes of reference HIV-1 strains representing subtypes A1, A2, B, C, D, and G as well as CRF01_AE (01_AE), CRF02_AG (02_AG), CRF15_01B (15_01B), CRF33_01B (33_01B), and CRF34_01B (34_01B) were included in the analyses (shown in bold letters). Sequence IDs are presented as a GenBank accession number, sample ID, or the ID of the reference HIV-1 strain, and the subtype or CRF of the reference strain (shown in parentheses) in that order. Bootstrap values were shown if they were >70.

individuals (24.3%) on long-term ART. Major drug resistance mutations detected in RT genes were E138G (33.3%), K103N (22.2%), and M184V (22.2%). Meanwhile, minor drug resistance mutations detected in RT genes were V106I (33.3%), V90I (11.1%) and V179D (11.1%) (**Table 2**).

A Major and Several Minor Drug Resistance Mutations were Detected in PR Genes

Protease inhibitor is used for the second-line regimens in combination with 2 NRTI drugs in Indonesia; however, no study participants in the present study were on ART with protease inhibitor. Therefore, drug resistance mutations



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in PR genes were studied to find TDR in this study. A major drug resistance mutation, I50V, was found in PR gene from a study participant. This major mutation is correlated with the resistance to darunavir/ritonavir (DRV/r), lopinavir/ritonavir (LPV/r), and fosamprenavir/ritonavir (**Table 3**). In addition, 12 minor drug resistance mutations were detected. Especially, minor mutations, M36I/K, H69K and L89M/I/V, were detected in all samples (**Table 3**).

DISCUSSION

The dominant HIV-1 subtype, CRF01_AE prevalent in Medan, was consistently detected in Jakarta, Bali, East Java, West Nusa Tenggara and other parts of Indonesia in previous

Table 2. Genotypic Characteristics and Drug Resistance Mutations Detected in RT Genes Derived from 9 Individuals on ART in Medan.

Sample	Subtype*	ART status	Drug Resistance Mutations**		Drug Resistance
			nRTIs	NNRTIs	
MED 11	CRF02_AG***	3TC, AZT, EFV		V90I	ETR
MED 12	CRF01_AE	TDF 3TC, EFV		V106I	DOR, ETR
MED 17	CRF01_AE	3TC, AZT, NVP	A62V**** K65R M184V	K103N E138G	ABC, FTC, 3TC TDF, ddI, d4T, EFV, NVP, RPV, ETR
MED19	CRF01_AE	3TC, AZT, EFV		E138G	ETR, RPV
MED 25	CRF01_AE	TDF, 3TC, NVP		E138A	ETR, RPV
MED 42	CRF01_AE	TDF, 3TC, EFV		K103N V106I	EFV, NVP, DOR, ETR
MED 46	CRF01_AE	TDF, 3TC, NVP		V106I	DOR, ETR
MED 48	CRF01_AE	3TC, AZT, NVP		E138G V179D	ETR, RPV
MED 65	CRF01_AE	3TC, AZT, NVP	M184V	Y181C	ABC, FTC, 3TC, EFV, ETR, NVP, RPV

* The subtype of RT genes was assigned based on RIP and phylogenetic analyses.
**The determination of drug resistance mutations was based on the guidelines published by the International Antiviral Society-United States (IAS-USA).
***Recombinants CRF02_AG
****The letter written in bold was showing major mutation.

Table 3. Genotypic Characteristics and Drug Resistance Mutations Detected in PR Genes Derived from 32 Individuals on ART in Medan.

Mutation*	Frequency (%)		
	all (n=32)	CRF01_AE** (n=30)	CRF02_ AG*** (n=2)
L10I/V	4 (12.5)	4 (13.3)	0 (0)
G16E	6 (18.8)	6 (20.0)	0 (0)
K20R/I	23 (71.9)	22 (73.3)	1 (50.0)
M36I/K	32 (100)	32 (100)	0 (0)
I50V****	1 (3.1)	1 (3.3)	0 (0)
I62V	5 (15.6)	5 (16.7)	0 (0)
L63P	5 (15.6)	3 (10.0)	2 (100)
I64V	1 (3.1)	0 (0)	0 (0)
H69K	32 (100)	30 (100)	2 (100)
V77I	10 (31.3)	10 (33.3)	0 (0)
V82I	2 (6.3)	2 (6.7)	0 (0)
L89M/I/V	32 (100)	30 (100)	2 (100)
I93L/M	5 (15.6)	4 (13.3)	1 (50.0)

*The determination of drug resistance mutations was based on the guidelines published by the International Antiviral Society United States (IAS-USA).
** The subtype of PR genes was assigned based on RIP and phylogenetic analyses.
*** Recombinants CRF02_AG
****The letter written in bold was showing major mutation.

studies.[6,11–14] Different transmission routes could account for the transmission of different HIV-1 subtypes. Previous studies by Merati et al,[14] 20128 showed that CRF01_AE was more frequently found among individuals with IDU rather than individuals with other transmission routes in Indonesia. In contrast, subtype B were more frequently found among individuals with the heterosexual transmission. However, interestingly in our results, recombinant viruses containing CRF01_AE gene fragment, rather than B subtype, were frequently detected among individuals with the heterosexual transmission. Our results were also in accordance with our previous results showing that CRF01_AE were frequently detected among female sex workers.[15]

Another recombinant form, CRF02_AG, was found in this study. Interestingly, the emergence of CRF02_AG was also detected in other parts of Indonesia in recent studies.[16–18] CRF02_AG was first detected from frozen serum samples collected in the Democratic Republic of the Congo (former Zaire) in 1976.[19] Similarity could be found between CRF01_AE and CRF02_AG throughout their genome, and both of them were rapidly and largely transmitted by heterosexual transmission.[20] However, its appearance in Indonesia needs to be further studied in order to reveal its role in the HIV-1 epidemic in Indonesia.

Among the HIV-1 subtype, the difference in cellular tropism or coreceptor usage, CCR5 (R5) and CXCR4 (X4), was reported,[21,22] and it was related to HIV-1 pathogenesis. Studies on coreceptor usage have been extensively conducted on HIV-1 subtype B and C, but not for other subtypes and CRFs. Therefore, further researches are required on subtypes and recombinants detected in this study. Generally, R5 viruses were detected over the entire course of disease progression after HIV-1 infection while X4 viruses were usually found in the late stage of disease progression. X4 vir uses were found in the late stage of disease progression for most HIV-1 subtypes except subtype C. The switch in coreceptor usage between R5 and X4 was correlated with faster CD4+-T cell decline and rapid development to AIDS.[23–25]

Acquired drug resistance mutations were detected in RT genes (24.32%). This number was consistent with those in our previous results in Bali, Maumere, and Kepulauan Riau.[6,13,17] Drug resistance mutations among individuals on ART have been detected in many other Asian countries such as Nepal[26] and China.[27] Major drug resistance mutations found in RT genes were correlated with the resistance against first-line regimens of ART in Indonesia. Drug resistance mutations against nucleoside RT inhibitors (NRTIs), ABC, FTC, 3TC, and TDF were detected in 2 out of 9 individuals while the mutations against non-nucleoside RT inhibitors (NNRTIs), ETR, RVP, EFV, and NVP were detected among 9 all individuals. ART-experienced individuals failing first-line ART regimen were subjected to second-line regimens, which is TDF or AZT, 3TC or FTC with LPV/r.

Although no study participants were on ART with protease inhibitors, we investigated drug resistance mutations in PR genes as TDR. A major drug mutation, I50V, was detected in a PR gene. The emergence of TDR potentially compromises second-line ART regimens in Indonesia; thus, we believe it is important to continue monitoring the appearance of TDR in PR genes in a future study. Minor drug resistance mutations detected in PR genes were M36I, H69K, and L89I. Those mutations were associated with natural polymorphisms detected among CRF01_AE viruses.[28]

CONCLUSION

The dominant HIV-1 subtype found in Medan, South Sumatera, was CRF01_AE. Several other subtypes and recombinant viruses including CRF02_AG were also detected. In addition, antiretroviral drug resistance mutations were observed in RT and PR genes. Resistance mutations against RT inhibitors were found in 24.3% of ART-experienced individuals. We also detected a major mutation in a PR gene (3.1%). Our results suggest the importance of continuous surveillance studies on HIV-1 subtypes and drug resistance mutations among ART-experienced individuals.

REFERENCES

1. RI DPKK. Laporan perkembangan HIV AIDS dan PIMS di Indonesia. 2018. Available from http://icjr.or.id/data/wp-content/uploads/2019/08/Penanggulangan-HIV-dalam-Ancaman-R-KUHP.pdf.
2. World Health Organization (WHO). Update of first and second line antiretroviral regimens. WHO. 2019. Available from https://apps.who.int/iris/bitstream/handle/10665/325892/WHO-CDS-HIV-19.15-eng.pdf?ua=1.
3. Hong SY, Nachega JB, Kelley K, et al. The global status of HIV drug resistance: clinical and public-health approaches for detection, treatment and prevention. Infect Disord Drug Targets. 2011;11(2):124–33.
4. Hosseinipour MC, Gupta RK, Van Zyl G, et al. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. J Infect Dis. 2013;207 Suppl 2:S49–56.
5. Takuva S, Louwagie G, Zuma K, et al. Durability of first line antiretroviral therapy: Reasons and predictive factors for modifications in a Swaziland Cohort. J Antivirals Antiretrovir. 2012;4(1):014–20.
6. Indriati DW, Kotaki T, Khairunisa SQ, et al. Appearance of drug resistance mutations among the dominant HIV-1 subtype, CRF01_AE in Maumere, Indonesia. Curr HIV Res. 2018;16(2):158-66.
7. Khairunisa S, Kotaki T, Witaningrum AM, et al. Appearance of drug resistance-associated mutations in human immunodeficiency virus type 1 protease and reverse transcriptase derived from drug-treated Indonesian patients. AIDS Res Human retroviruses. 2015;31(2):255–9.
8. Merati TP, Ryan CE, Spelmen T, et al. CRF01-AE dominates the HIV-1 epidemic in Indonesia. Sex Health. 2012;9(5):414–21.
9. Wensing AM, Calvez V, Ceccherini-Silberstein F, et al. 2019 update of the drug resistance mutations in HIV-1.

Top Antivir Med. 2019;27(3):111-21.
10. Porter KR, Mascola JR, Hupudio H, et al. Genetic, antigenic and serologic characterization of human immunodeficiency virus type 1 from Indonesia. J Acquir Immune Defic Syndr. 1997;14(1):1-6.
11. Witaningrum AM, Khairunisa SQ, Ueda S, et al. Viral subtyping of HIV-1 derived from infected, drug-naive individuals in Jakarta, Indonesia. 2018 IOP Conf Ser: Mater Sci Eng. 2018;434:012321.
12. Sahbandar IN, Takahashi K, Djoerban Z, et al. Current HIV type 1 molecular epidemiology profile and identification of unique recombinant forms in Jakarta, Indonesia. AIDS Res Hum Retroviruses. 2009;25(7):637–46.
13. Khairunisa SQ, Masyeni S, Witaningrum AM, et al. Genotypic characterization of human immunodeficiency virus type 1 isolated in Bali, Indonesia in 2016. HIV AIDS Rev. 2018;17(2):81-90.
14. Merati TP, Ryan CE, Spelmen T, et al. CRF01_AE dominates the HIV-1 epidemic in Indonesia. Sex Health. 2012;9(5):414–21.
15. Kotaki T, Khairunisa SQ, Sukartiningrum SD, et al. High Prevalence of HIV-1 CRF01_AE viruses among female commercial sex workers residing in Surabaya, Indonesia. PLoS ONE. 2013;8(12):e82645.
16. Indriati DW, Kamasawa N, Matsui K, et al. Quantitative localization of Cav2.1 (P/Q-type) voltage-dependent calcium channels in Purkinje cells: somatodendritic gradient and distinct somatic coclustering with calcium-activated potassium channels. J Neurosci. 2013;33(8):3668–78.
17. Khairunisa SQ, Ueda S, Witaningrum AM, et al. Genotypic characterization of human immunodeficiency virus type 1 prevalent in Kepulauan Riau, Indonesia. AIDS Res Hum Retroviruses. 2018;34(6):555-60.
18. Ueda S, Witaningrum AM, Khairunisa SQ, et al. Genetic diversity and drug resistance of HIV-1 circulating in North Sulawesi, Indonesia. AIDS Res Hum Retroviruses. 2018;35(4):407–13.
19. Choi DJ, Dube S, Spicer TP, et al. HIV type 1 isolate Z321, the strain used to make a therapeutic HIV type 1 immunogen, is intersubtype recombinant. AIDS Res Hum Retroviruses. 1997;13(4):357–61.
20. Cornelissen M, Van Den Burg R, Zorgdrager F, et al. Spread of distinct human immunodeficiency virus type 1 AG recombinant lineages in Africa. J Gen Virol. 2000;81(2):515–23.
21. Dragic T, Litwin V, Allaway GP, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature. 1996;381(6584):667–73.
22. Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 1996;272(5263):872–7.
23. Koot M, Keet IP, Vos AH, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med. 1993;118(9):681–8.
24. Karlsson A, Parsmyr K, Sandstrom E, et al. MT-2 cell tropism as prognostic marker for disease progression in human immunodeficiency virus type 1 infection. J Clin Microbiol. 1994;32(2):364–70.
25. Esbjörnsson J, Månsson F, Martínez-Arias W, et al. Frequent CXCR4 tropism of HIV-1 subtype A and CRF02_AG during late-stage disease - indication of an evolving epidemic in West Africa. Retrovirology. 2010;7:1–13.
26. Bhusal N, Sutthent R, Horthongkham N, et al. Prevalence of HIV-1 subtypes and antiretroviral drug resistance mutations in Nepal. Curr HIV Res. 2016;14(6):517-24.
27. Luo XL, Mo LD, Su GS, et al. Incidence and types of HIV-1 drug resistance mutation among patients failing first-line antiretroviral therapy. J Pharmacol Sci. 2019;139(4):275–9.
28. Iemwimangsa N, Pasomsub E, Sukasem C, et al. Surveillance of HIV-1 drug-resistance mutations in Thailand from 1999 to 2014. Southeast Asian J Trop Med Public Health. 2017;48(2):271–81.