Identification of HIV-1 subtypes and drug resistance mutations among HIV-1-infected individuals residing in Pontianak, Indonesia

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Abstract

Introduction **The present study investigated the HIV-1 subtype classification in addition to prevalence of drug resistance mutations (DRMs) in antiretroviral therapy (ART)-experienced and ART-naïve residents of Pontianak, West Kalimantan, Indonesia.**

Methods **Whole blood samples collected from 30 HIV-1-infected individuals, comprising 19 ART-experienced and 11 ART-naïve individuals, were subjected to RNA and DNA extraction, followed by HIV-1 genes amplification and sequencing analysis. HIV-1 subtyping was classified on viral *pol* genes encoding reverse transcriptase (RT gene) and protease (PR gene) accompanied by the *env* and *gag* genes. DRMs in the RT and PR genes were also analyzed.**

Results **CRF01_AE was identified as the predominant circulating recombinant form (CRF) of HIV-1 in both ART-experienced and ART-naïve individuals. In addition, CRF02_AG, subtype B, recombinant virus expressing CRF01_AE and subtype B viral genomic fragments, also recombinant virus containing CRF01_AE and CRF02_AG genomic fragments were also identified. Acquired drug resistance (ADR) was identified in 28.5% of ART-experienced individuals, while no transmitted drug resistance was identified in ART-naïve individuals.**

Conclusions **This study identified CRF01_AE as the most predominant HIV-1 CRF distributing in Pontianak, Indonesia. The prevalence of ADR is considered to be high; thus, further surveillance is needed in this region.**

Keywords **HIV-1, CRF01_AE, acquired drug resistance, transmitted drug resistance, Indonesia**

Introduction

Indonesia is currently ranked as the fourth country with the highest population in the world, and the fourth country counted for the new human immunodeficiency virus (HIV) infection cases annually. As stated by the report of the Joint United Nations Program on HIV/AIDS, Indonesia recorded about 48,000 (in a range between 43,000-52,000) novel HIV positive cases and 38,000 (in a range between 34,000-43,000) AIDS-related deaths in 2016.[1] The Ministry of Health Republic Indonesia estimated that only

Received: 10 March 2020; revised: 15 July 2020; accepted: 20 July 2020.

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Published September 2020

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

11-15% of HIV-positive Indonesians were on antiretroviral therapy (ART) by the end of 2016. Furthermore, provincial estimates of HIV prevalence range between 0.1 and more than 2.0% in Indonesia.[2]

According to HIV epidemiological data of Indonesia in 2016, Indonesian provinces with a high HIV prevalence rate and high response by the provincial government in dealing with HIV cases were Jakarta, East Java, and Bali provinces; provinces with a high HIV prevalence rate and relatively low response were Papua and West Kalimantan provinces; and the province with a low HIV prevalence rate and relatively low response was Maluku province. Three cities, Pontianak, Singkawang, and Singkang, were acknowledged to have the most prominent HIV prevalence rate in West Kalimantan.[2]

Pontianak is the capital city of West Kalimantan and developed as the trading port of Borneo Island. It is located on the equator; hence, it is widely known as Kota Khatulistiwa (an equatorial city). In terms of its population, the number of residents in Pontianak has been progressively expanding each year. The population growth rate between 2013 and 2017 was approximately 6.79%.[3] Due to high rates of the HIV epidemic and its crucial functions as a trading port of Borneo Island, HIV-1 epidemiology in Pontianak needs to be examined in more detail. Previous studies reported CRF01_AE as the most predominant HIV-1 circulating recombinant form (CRF) prevalent in most Indonesian regions, including the East Java, Bali, Kepulauan Riau, and Nusa Tenggara provinces, while subtype B was highly prevalent in the Papua and West Papua provinces.[4-9] However, the information about HIV-1 epidemiology in Pontianak remains deficient.

Along with the identification of HIV-1 subtypes and CRFs, identification of drug resistance mutations (DRMs) is also essential. ART treatment has been proven to significantly enhance the quality of life of HIV-infected individuals and noteworthy ART has also reduced the mortality and morbidity cases associated with HIV-1 infection. The presence of acquired drug resistance (ADR) in ART-experienced patients and transmitted drug resistance (TDR) in ART-naïve patients may have undesirable effects on the clinical manifestation of HIV infection. The incidence of genotypic DRMs among ART-naïve, HIV-positive pregnant women was approximated in a range of 2.3–25%, and was currently reported to be 24% in newly infected juveniles. In Surabaya, East Java, the prevalence of TDR was found to be less than 5%, signifying that the current established first-line regimen remains efficient.[4]

In the present study, we accomplished genotypic analyses on HIV-1 *pol* genes encoding protease (PR gene) and reverse transcriptase (RT gene). Additionally, the *env* and *gag* genes originated from ART-experienced and ART-naïve patients residing in Pontianak, West Kalimantan, Indonesia were also studied. HIV-1 subtype distributions and the prevalence of both ADR and TDR in this region were examined herein.

Methods

Research participants and sample collection

Whole blood samples were obtained at the Voluntary Counseling and Testing (VCT) program in Pontianak Hospital. Thirty HIV-1-infected individuals, including 19 ART-experienced and 11 ART-naïve individuals, were enrolled in this study. Inclusion criteria of study participants were: age 18 or above, HIV-infected, as well as ART-experienced individuals who had received ART for more than one year, or ART-naïve individuals who had never received the treatment, while exclusion criteria were age <18 and pediatric patients. Thirty study participants were randomly selected. Ten milliliters of whole blood samples were stored in ethylenediaminetetraacetic acid (EDTA)-treated vacutainer tubes individually from all participants. Plasma separation was accomplished by centrifugation at 2,000 rpm for 10 min. In addition, peripheral blood mononuclear cells (PBMCs) were collected by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO, USA). The plasma specimen from ART-naïve individuals were then subjected to RNA isolation using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany), while PBMCs from ART-experienced individuals were subjected to DNA isolation utilizing the

QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany).

Amplification of HIV-1 genomic fragments and sequencing analysis

Firstly, viral RNA was reverse transcribed into cDNA by the use of SuperScript III First-Stand Synthesis kit (Invitrogen, Carlsbad, CA, USA) and the reverse primer, K-env-R1, 5'-CCAATCAGGGAAGAAGCCTTG-3' [corresponding to nucleotides (nt) 9168 to 9148 of a HIV-1 reference strain, HXB2 (GenBank accession no. K03455), HXB2 numbering]. The HIV-1 PR, RT, *env*, and *gag* genes were amplified by using GoTaq green master mix (Promega, Madison, WI, USA) and specific primer sets corresponding to the targeted genes. All primers are described in Table 1. Detailed PCR conditions are accessible upon request. PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The PCR products were then subjected to a sequencing analysis in order to assess the genomic fragment of the predominant viral population in a sample. A sequencing analysis of the amplified HIV-1 genomic fragment was assessed by using BigDye Terminator v3.1 Cycle Sequencing kit specifically provided for ABI PRISM 3500 xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were collected and aligned using Genetyx software version 10 (Genetyx, Tokyo, Japan). The collection of nucleotide sequences of targeted HIV-1 gene fragments has been submitted to the GenBank database under the following accession numbers, MN518004 - MN518016 (PR genes), MN518017 - MN518027 (RT genes), MN518039 - MN518054 (*env* genes), and MN518028 - MN518038 (*gag* genes).

HIV-1 subtyping and phylogenetic analysis

HIV-1 subtyping was classified using the recombinant identification program (RIP) and jumping profile Hidden Markov Model (jpHMM)-HIV (http://jphmm.gobics.de/submission_hiv). In addition, neighbor-joining (NJ) trees with a Kimura two-parameter model were generated by MEGA 6.06 software. Subtypes A1, A2, B, C, D, and G as well as CRF01_AE and CRF02_AG are considered to be major pandemic strains of HIV-1, while 3 CRF01_AE/subtype B-recombinants, CRF15_01B, CRF33_01B, and CRF34_01B, were detected frequently in Indonesia. Therefore, reference strains representing these subtypes and CRFs were retrieved from HIV database (https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/RefSeqs2005/RefSeqs05.html), and included in the phylogenetic analysis. HIV-1 subtyping was identified according to the successfully sequenced PR, RT, *gag* and/or *env* genes, and if the discrepancy in the subtype or CRF among those genes existed, the viral gene was accounted from a recombinant form of HIV-1.

HIV drug resistance analysis

The evaluation of drug resistance-related mutations in RT and PR genes originated from ART-experienced and ART-naïve individuals was manually assessed based on the guidelines issued by the International Antiviral Society-United States (IAS-USA).[10] In addition, the data was verified by using online Genotypic Resistance Interpretation Algorithm (https://hivdb.stanford.edu/hivdb/by-mutations/).

Ethics declaration

Ethical clearance was acquired from the Institutional Ethics Committees of Universitas Airlangga (approval number: 25-995/UN3.14/PPd/2013) and Kobe University Graduate School of Medicine (approval number: 784). Inscribed informed consents were collected from all research participants prior to admission in this study.

Results

Demographic and clinical data of study participants

Among the 30 individuals enrolled in this study, 19 were already under ART for more than one year, while 11 were ART-naïve. The most commonly used ART regimen was the combination of lamivudine (3TC), zidovudine (AZT), and nevirapine (NVP) (36.7%), with an

Table 1. Primers used for nested PCR

Genes		Primer name	Primer locations (HXB2 numbering)	Sequences 5' - 3'
PR	1st round	DRPR05	nt 2074 to 2095	AGACAGGYTAATTTTTTAGGGA
		DRPR02L	nt 2716 to 2691	TATGGATTTTCAGGCCCAATTTTTGA
	2nd round	DRPR01M	nt 2148 to 2167	AGAGCCAACAGCCCCACCAG
		DRPR06	nt 2611 to 2592	ACTTTTGGGCCATCCATTCC
RT	1st round	RT1L	nt 2388 to 2410	ATGATAGGGGGAATTGGAGGTTT
		DRRT4L	nt 4402 to 4380	TACTTCTGTTAGTGCTTTGGTTCC
	2nd round	RT7L	nt 2485 to 2509	GACCTACACCTGTCAACATAATTGG
		DRRT6L	nt 4309 to 4285	TAATCCCTGCATAAATCTGACTTGC
env	1st round	M5	nt 6858 to 6889	CCAATTCCCATACATTATTGTGCCCCAGCTGG
		M10	nt 7661 to 7632	CCAATTGTCCCTCATATCTCCTCCTCCAGG
	2nd round	M3	nt 6948 to 6973	GTCAGCACAGTACAATGIACACATGG
		M8	nt 7547 to 7521	TCCTTCCATGGGA GGGGCATACATTGC
gag	1st round	H1G777	nt 1231 to 1255	TCACCTAGAACTTTGAATGCATGGG
		H1P202	nt 2352 to 2325	CTAATACTGTATCATCTGCT GCTCCTGT
	2nd round	H1Gag1584	nt 1577 to 1595	AAAGATGGATAATCCTGGG
		G17	nt 2040 to 2017	TCCACATTTC CAACAGCCCTTTTT

average duration of treatment of more than 3 years. The median age of study participants was 33.8 years, with the ART-experienced and ART-naïve groups being 33.9 years and 33.5 years respectively. Eighty percent of study participants were male, while 20% were female. All participants were married, and the major risk behavior for HIV-1 infection was intravenous drug use (66.7%, 20/30). Seventeen (89.5%) ART-experienced and all (100%) ART-naïve individuals were diagnosed with opportunistic infections. The demographic and clinical data of study participants are shown in Table 2.

HIV-1 subtyping

Phylogenetic trees were generated using efficiently sequenced 13 PR (296 bp), 11 RT (762 bp), 16 *env* (383 bp), and 9 *gag* gene fragments (369 bp) obtained from 26 samples (Figure 1). For ART-naïve samples, we first attempted to amplify viral gene fragments from cDNA synthesized from RNA isolated from plasma samples; however, it failed. Therefore, all viral gene fragments were amplified from DNA isolated from PBMCs derived from ART-naïve and ART-experienced individuals (data not shown). Among ART-experienced individuals, 10/16 (62.5%) were found to be infected with CRF01_AE, while 2/16 (12.5%), 1/16 (6.3%), 1/16 (6.3%), and 2/16 (12.5%) were infected with CRF02_AG, subtype B, recombinant virus containing CRF01_AE and subtype B genomic fragments, and recombinant virus containing CRF01_AE and CRF02_AG genomic fragments, respectively. Among ART-naïve individuals, the HIV-1 subtypes and CRFs detected were CRF01_AE (50%, 5/10), CRF02_AG (30%, 3/11), and subtype B (20%, 2/10). Subtyping results are summarized in Table 3.

Prevalence of acquired drug resistance (ADR) and transmitted drug resistance (TDR)

Seven PR and 4 RT gene sequences originated from ART-naïve individuals, while 6 PR and 7 RT gene sequences were obtained from ART-experienced individuals. DRMs were identified in the PR and RT genes as stated by the IAS-USA guidelines[10] as well as detected by online Genotypic Resistance Interpretation Algorithm (https://hivdb.stanford.edu/hivdb/by-mutations/). The major DRMs remained unidentified in the PR genes, while minor DRMs were recurrently detected. All PR genes obtained from ART-naïve and ART-experienced individuals contained at least one minor DRM. Minor mutations also frequently detected in PR genes were M36I (100%, 13/13), K20I/R

Table 2. Demographic and clinical information on ART-experienced and ART-naïve individuals residing in Pontianak

Characteristics	Total	On ART	Non-ART
Sample number	30	19	11
Age, mean years (SD)	33.8 (6.2)	33.9	33.5
Sex, n (%)			
Male	24 (80.0%)	18 (94.7%)	6 (54.5%)
Female	6 (20.0%)	1 (5.3%)	5 (45.5%)
Marital status, n (%)			
Married	30 (100.0%)	19 (100.0%)	11 (100.0%)
Single	0 (0.0%)	0 (0.0%)	0 (0.0%)
Transmission risk category, n (%)			
Heterosexual intercourse	7 (23.3%)	2 (10.5%)	5 (45.5%)
Intravenous drug use	20 (66.7%)	17 (89.5%)	3 (27.3%)
Homosexual intercourse	3 (10.0%)	0 (0.0%)	3 (27.3%)
Types of ART used, n (%)			
3TC+AZT+NVP	11 (36.7%)	11 (57.9%)	0 (0.0%)
3TC+AZT+EFV	2 (6.7%)	2 (10.5%)	0 (0.0%)
Others	6 (20.0%)	6 (31.6%)	0 (0.0%)
Duration of ART, n (%)			
<1 year	0 (0%)	0 (0.0%)	0 (0.0%)
1-3 years	2 (6.7%)	2 (10.5%)	0 (0.0%)
>3 years	17 (56.7%)	17 (89.5%)	0 (0.0%)
Opportunistic infection, n (%)			
No	2 (6.7%)	2 (10.5%)	0 (0.0%)
Yes	28 (93.3%)	17 (89.5%)	11 (100.0%)

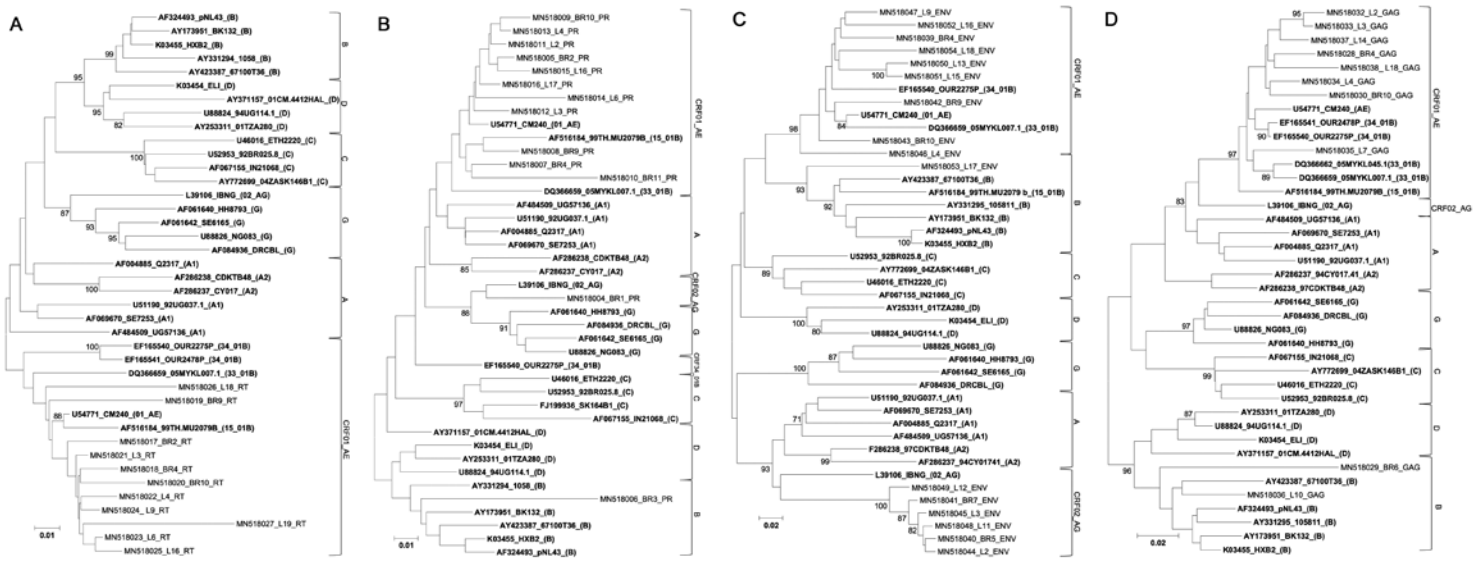
(69.2%, 9/13), and V77I (38.5%, 5/13) (data not shown).

No TDR was identified among ART-naïve individuals; however, ADR in the RT genes was identified in two out of seven ART-experienced individuals (28.6%) (Table 4). Three nucleoside RT inhibitor (NRTI)-associated DRMs (K65R, M184V, K219E) and 4 non-nucleoside RT inhibitor (nNRTI)-related DRMs (K130T, Y181C, G190A and P225H) were detected. These mutations confer resistance to abacavir (ABC), didanosine (ddI), lamivudine (3TC), stavudine (d4T), emtricitabine (FTC), tenofovir (TDF), zidovudine (AZT), nevirapine (NVP), etravirine (ETR), efavirenz (EFV), and rilpivirine (RPV).[11]

Discussion

In the present study, CRF01_AE was identified as the major CRF of HIV-1 circulating in Pontianak, West Kalimantan, Indonesia. This result was consistent with previous findings obtained in other Indonesian regions, including the East Java, Bali, Kepulauan Riau, and Nusa Tenggara provinces.[4-9] CRF01_AE is also known to be predominant in Southeast-Asian countries, including Thailand, the Philippines, Singapore, and Vietnam,[12,13] along with East Asian countries, consisting of Japan, China including Hong Kong and Taiwan, and South Korea.[12-14] In addition, CRF01_AE was reported to be the second largest CRF circulating worldwide, with an estimated global prevalence rate of 5.3%.[14] HIV-1 CRF01_AE infection correlated with a rapid CD4 decline, higher viral load, and significant risk of accelerated disease progression in China.[15,16] Therefore, the consecutive and periodic surveillance of HIV-1 subtypes is fundamental to be accomplished due to the impact of different subtypes and CRFs on disease progression.

The presence of DRMs compromises the effectiveness of ART, thereby hindering treatment success.[17] Several mutations in the



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.

Figure 1. Phylogenetic tree analysis of HIV-1 RT, PR, *env*, and *gag* gene sequences collected in Pontianak, Indonesia

HIV-1 RT genes have been shown to reduce viral susceptibility to ART.[18] The manifestation of TDR among ART-naïve individuals resulted from the acquisition of a drug-resistant virus.[19] Our previous studies in Surabaya, West Papua, and Bali revealed the occurrence of TDR in RT inhibitors counted for 4.3% (2/47), 12.9% (4/31), and 16.7% (5/30) of RT genes obtained from ART-naïve individuals, respectively.[4,8-9] In other countries, various prevalence rates of TDR were identified, including 17.2% in Germany, 12% in South Carolina, USA, 20.5% in Washington DC, USA, and 3.6% in China.[20-23] However, no TDR was observed among ART-naïve individuals residing in Pontianak in the present study. This result indicates that the current first-line regimen is still effective for newly infected individuals in the region.[4]

In contrast, ADR of the RT inhibitor was identified in 28.5% of ART-experienced individuals in Pontianak, which is considered to be high. Previous studies in Bali, Maumere, and Kepulauan Riau also identified ADR of the RT inhibitor among ART-experienced individuals.[5,6] A high prevalence of ADR was also identified in other countries, such as Washington DC, USA (40.5%), and Cameroon (17.7-28.3%).[22-24]

Among two individuals presenting major DRMs towards an RT inhibitor, one individual (L19) was infected with HIV-1 variant(s) containing a P255H mutation, which correlated with resistance towards EFV. Another individual (L18) was infected with HIV-1 variant(s) containing several mutations that associated with resistance towards multiple drugs such as FTC, ddI, ABC, 3TC, d4T, TDF, ETR, EFV, NVP, AZT, and RPV.[10] Switching to other antiretroviral drugs or regimens may be favorable for individuals with ADR in order to maintain the effectiveness of treatment.

In ART-experienced and ART-naïve individuals, no major DRMs related to PR inhibitors were identified in Pontianak. The result is consistent with previous findings reporting the absence of both ADR and TDR related to PR inhibitors in several Indonesian regions.[4-8] Among ART-experienced individuals in Indonesia, only 3.3% (2,983 of 91,369) were

Table 3. HIV-1 subtyping

Sample ID	Treatment	Subtype/CRF assignment	Subtyping			
			PR gene	RT gene	Env gene	Gag gene
BR1	Non-ART	CRF02_AG*	CRF02_AG	NA**	NA	NA
BR2	Non-ART	CRF01_AE	CRF01_AE	CRF01_AE	NA	NA
BR3	Non-ART	B	B	NA	NA	NA
BR4	Non-ART	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
BR5	Non-ART	CRF02_AG	NA	NA	CRF02_AG	NA
BR6	Non-ART	B	NA	NA	NA	B
BR8	Non-ART	NA	NA		NA	NA
BR7	Non-ART	CRF02_AG	NA	NA	CRF02_AG	NA
BR9	Non-ART	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
BR10	Non-ART	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
BR11	Non-ART	CRF01_AE	CRF01_AE	NA	NA	NA
L1	On ART	NA	NA	NA	NA	NA
L2	On ART	CRF01_AE	CRF01_AE	NA	CRF02_AG	CRF01_AE
L3	On ART	CRF01_AE	CRF01_AE	CRF01_AE	CRF02_AG	CRF01_AE
L4	On ART	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
L5	On ART	NA	NA	NA	NA	NA
L6	On ART	CRF01_AE	CRF01_AE	CRF01_AE	NA	NA
L7	On ART	CRF01_AE	NA	NA	NA	CRF01_AE
L8	On ART	NA	NA	NA	NA	NA
L9	On ART	CRF01_AE	NA	CRF01_AE	CRF01_AE	NA
L10	On ART	B	NA	NA	NA	B
L11	On ART	CRF02_AG	NA	NA	CRF02_AG	NA
L12	On ART	CRF02_AG	NA	NA	CRF02_AG	NA
L13	On ART	CRF01_AE	NA	NA	CRF01_AE	NA
L14	On ART	CRF01_AE	NA	NA	NA	CRF01_AE
L15	On ART	CRF01_AE	NA	NA	CRF01_AE	NA
L16	On ART	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
L17	On ART	CRF01_AE/B***	CRF01_AE	NA	B	NA
L18	On ART	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
L19	On ART	CRF01_AE	NA	CRF01_AE	NA	NA

*Recombinant form of HIV-1 containing the viral gene fragments of subtypes A and G.
**Not available due to the failure of PCR.
***Recombinant form of HIV-1 containing the viral gene fragments of CRF01_AE and subtype B.

receiving a PR inhibitor-based ART regimen in 2017.[25] The limited use of a PR inhibitor-based regimen may contribute to the absence of DRMs related to PR inhibitors. Although no major mutations were found, several minor mutations were identified in the PR genes, including M36I, K20R, V77I, I93L, and G16E. These mutations have been identified as natural polymorphisms among CRF01_AE viruses.[17] Finally, the present study had the following limitations. Thirty

Table 4. Appearance of drug resistance mutations among ART-experienced individuals

Sample ID	ART regimen	Drug resistance mutations*		
		NRTI	NNRTI	Confer resistance to
L18	AZT+3TC+NVP TDF+3TC+NVP	**K65R** **M184V** **K219E**	- **Y181C** **G190A**	ABC, ddI, FTC, 3TC, d4T, TDF, AZT, EFV, ETR, NVP, RPV
L19	3TC+AZT+NVP	-	**K130T** **P225H**	**EFV**

*Drug resistance mutations causing high resistance to antiretrovirals according to the guidelines published by the International Antiviral Society United States (IAS-USA) as well as detected by online Genotypic Resistance Interpretation Algorithm (https://hivdb.stanford.edu/hivdb/by-mutations/) are shown. Drug resistance-associated major mutations are shown in bold.

ABC – abacavir; **ART** – antiretroviral therapy; **AZT** – zidovudine; **d4T** – stavudine; **ddI** – didanosine; **EFV** – efavirenz; **ETR** – etravirine; **FTC** – emtricitabine; **NNRTI** – non-nucleoside reverse transcriptase inhibitor; **NRTI** – nucleoside reverse transcriptase inhibitor; **NVP** – nevirapine; **TDF** – tenofovir; **RPV** – rilpivirine; **3TC** – lamivudine.

individuals were enrolled in this study, and the sample size may not be sufficiently large to provide clear research outcomes. It was due to a difficulty in recruiting more HIV-infected individuals at the site during sampling duration. In addition, we faced a low success rate of PCR amplification for viral partial gene fragments. It might be explained by sample storage and the transportation under improper conditions. Nevertheless, information on HIV epidemiology was quite limited in Pontianak, Indonesia, and we believe the data shown in the present study provide important information for revealing the situation of HIV infection in the region.

Conclusions

CRF01_AE was identified as the dominant CRF circulating in Pontianak, West Kalimantan, Indonesia. Several other subtypes identified included CRF02_AG, subtype B, recombinant virus containing CRF01_AE and subtype B genomic fragments, and recombinant virus containing CRF01_AE and CRF02_AG genomic fragments. TDR against PR and RT inhibitors was not found in ART-naïve individuals; however, ADR against an RT inhibitor was found in 28.5% of ART-experienced individuals, which is considered to be high. These results suggest the significance of the regular surveillance of HIV-1 subtypes and DRMs in both ART-experienced and ART-naïve individuals.

Authors' contributions statement: MK and N conceived and supervised the study; SQK and TK designed experiments; DN organized study participants; SQK, NLAM and DWI conducted experiments; SQK and TK collected samples and clinical information; SQK and NLAM analyzed data; SQK wrote the manuscript; SQK and MK made manuscript revisions.

Conflicts of interest: All authors – none to declare.

Funding: Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) administered by the Ministry of Education, Culture, Sport, Science and Technology – Japan; the Japan Agency for Medical Research and Development (AMED); and the Institute of Tropical Disease as the Center of Excellence (COE) platform by the Ministry for Research and Technology (RISTEK) of Indonesia.

Acknowledgements: The authors would like to acknowledge all the study participants and the staff from Voluntary Counseling and Testing (VCT) program in Pontianak Hospital. Deepest gratitude also extends to J-GRID, AMED and ITD – Universitas Airlangga to facilitate this study. The manuscript was proofread by Medical English Service, Kyoto, Japan.

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Please cite this article as:
Khairunisa SQ, Megasari NLA, Indriati DW, Kotaki T, Natalia D, Nasronudin, Kameoka M. Identification of HIV-1 subtypes and drug resistance mutations among HIV-1-infected individuals residing in Pontianak, Indonesia. GERMS. 2020;10(3):174-183. doi: 10.18683/germs.2020.1203