Sero- and Molecular Epidemiology of HIV-1 in Papua Province, Indonesia

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ABSTRAK

Latar belakang: *infeksi* human immunodeficiency virus *(HIV) dan* acquired immune deficiency syndrome *(AIDS) merupakan masalah kesehatan masyarakat yang sangat serius dan mempengaruhi perekonomian di Indonesia. Provinsi Papua memiliki prevalensi HIV tertinggi di negeri ini. Namun data tentang epidemiologi HIV sangat terbatas. Oleh karena itu, dilakukan studi sero-epidemiologi HIV pada orang sehat yang berada di daerah Paniai, Papua.* ***Metode:*** *pemeriksaan serologi dilakukan pada 157 orang sehat dari populasi umum di Paniai, Papua. Selain itu, studi epidemiologi molekuler kemuian dilakukan pada gen HIV tipe 1 (HIV-1) dari individu yang ditemukan terinfeksi. Analisis genotipe dilakukan terhadap sampel darah perifer dari individu HIV-1-positif dan 15 sampel tambahan dari individu yang sebelumnya telah dikonfirmasi positif HIV-1.* ***Hasil:*** *pemeriksaan serologis menunjukkan bahwa 2 dari 157 orang sehat (1,27%) dinyatakan positif HIV. Pemeriksaan genotip pada sampel HIV-1 menunjukkan bahwa subtipe B dan* circulating recombinant form *(CRF) CRF01_AE merupakan subtipe terbanyak dan CRF HIV-1 yang banyak ditemukan di wilayah tersebut, sementara subtipe A1 dan rekombinan antara CRF01_AE dan subtipe B juga ditemukan pada penelitian ini. Selain itu, pada pasien HIV yang sudah mendapatkan terapi ARV juga ditemukan mutasi mayor pada daerah reverse transcriptase.* ***Kesimpulan:*** *hasil penelitian ini dapat memberikan informasi penting untuk memahami situasi perkembangan HIV/AIDS saat ini di provinsi Papua di Indonesia.*

Kata kunci: *HIV-1, Papua, subtipe B, CRF01_AE, resistensi obat HIV.*

ABSTRACT

Background: *human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) cause serious health problems and affect the Indonesian economy. Papua province has the highest prevalence of HIV infection in the country; however, epidemiological data are limited. Therefore, in order to*

reveal the current situation of HIV/AIDS in Papua province, sero- and molecular epidemiological studies of HIV were conducted. ***Methods:*** *serological tests were conducted on 157 healthy individuals from the general population residing in Paniai, Papua. In addition, a molecular epidemiological study was then conducted on HIV type 1 (HIV-1) genes derived from infected individuals. Peripheral blood samples from HIV-1-positive individuals and 15 additionally enrolled, previously confirmed HIV-1-positive individuals were subjected to a genotypic analysis.* ***Results:*** *serological tests revealed that 2 out of 157 (1.27%) healthy individuals were HIV-positive. In addition, HIV-1 subtyping revealed that subtype B and CRF01_AE were the major subtype and circulating recombinant form (CRF) of HIV-1 prevalent in the region, while subtype A1 and a recombinant form including viral gene fragments of CRF01_AE and subtype B was also detected. In addition, HIV drug resistance-associated major mutations were detected in the reverse transcriptase gene derived from infected individual on antiretroviral therapy.* ***Conclusion:*** *these results provide important information for clearer understanding on the current situation of HIV/AIDS in Papua province in Indonesia.*

Keywords: *HIV-1, Papua, subtype B, CRF01_AE, HIV drug resistance.*

INTRODUCTION

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). Although the number of new cases of HIV continues to decrease worldwide each year, the epidemic growth of HIV is continuing in several countries.[1] In Asia and the Pacific region, the annual incidence of HIV infection has decreased in many countries, including India, Myanmar, Thailand, Vietnam and Papua New Guinea; however it has continuously and markedly increased in Indonesia, particularly since 2000.[1,2] In 2013, there were 640,000 individuals with HIV type 1 (HIV-1) in Indonesia,[1,2] while the annual number of new cases of HIV in Indonesia was 75,000, accounting for 23% of all cases among countries in Asia and the Pacific region, second to India (38%).[1] Hence, HIV/AIDS remains a significant public health issue in Indonesia and has a negative impact on its economy. The largest number of individuals with HIV-1 is in Jakarta, the capital city of Indonesia, while the highest prevalence has been reported in Papua province.[3] The major route of HIV-1 infection is considered to be through heterosexual transmission in Papua, and key HIV-affected populations are aged between 15 and 49 years old. In addition, the prevalence of HIV among the ethnic Papuan population is almost 2-fold of that among non-ethnic Papuans (2.8% versus 1.5%).[4] Nevertheless, epidemiological data on HIV-1 in Indonesia, particularly in rural areas such as Papua province, are limited.

HIV-1 is characterized by extensive genetic heterogeneity and has been divided into four groups: M (major), O (outlying), N (new or non-M, non-O) and P (pending). Group M viruses are further classified into a number of subtypes and circulating recombinant forms (CRFs). Of these, subtypes A, B, C, D and G, as well as CRF01_AE and CRF02_AG, are the major subtypes and CRFs responsible for the worldwide HIV-1 pandemic.[5] While subtype B of HIV-1 is the predominant subtype in the Americas, Europe and Australia, there is a growing epidemic of non-B subtypes and CRFs in Africa and Asia. CRF01_AE is prevalent throughout Southeast Asia[5] and is responsible for more than 90% of infected cases in Indonesia.[6] In addition, several recombinant forms between CRF01_AE and subtype B, including CRF33_01B, have emerged in Indonesia.[6-9] Different subtypes and CRFs are considered to show different rates of disease progression, immune responses, responses to antiretroviral therapy (ART), and/or the development of drug resistance.[10] Therefore, it is important to monitor the global prevalence of subtypes and CRFs for HIV-1 prevention and control as well as for vaccine development.

In order to clarify the prevalence of HIV-1 in Papua province in Indonesia, a sero-epidemiological study of HIV-1 among 157 healthy individuals was conducted in Paniai, Papua. In addition, a molecular epidemiological study on the viral genomes derived from 17

HIV-1-infected individuals was also performed in order to identify currently circulating HIV-1 subtypes and CRFs in Papua.

METHODS

This study was conducted with approval from the Institutional Ethics Committees of the Institute of Tropical Disease and the Institute for Research and Public Service, Universitas Airlangga on September 2nd, 2013 with a reference number of 25-995/UN3.14/PPd/2013 and Kobe University Graduate School of Medicine on September 20th, 2012, as well as with written informed consent from study participants.

Study Participants and Sample Collection

In this study, we collected two sets of blood samples. The first set were collected from blood samples from general population for the sero-epidemiological study of HIV-1, while the second set were collected samples from HIV-1-infected individuals for the molecular epidemiological study of HIV-1 (**Figure 1**). In the sero-epidemiological study of HIV-1, 157 healthy individuals were enrolled from the general population with assistance from the Enarotali Public Health Center and the General Hospital of Paniai in August 2014. The samples from general population were obtained randomly from some patients who had previously diagnosed with viral hepatitis or some general patients coming to the Enarotali Public Health Center and the General Hospital of Paniai. Fifteen individuals previously diagnosed with HIV-1 seropositive were additionally enrolled at the General Hospital of Paniai for the molecular epidemiological study of HIV-1. Most participants were from indigenous tribes of Papua, while 12 participants were from other provinces in Indonesia. The personal information of study participants, including age and gender as well as the statuses of the Hepatitis B surface antigen (HBs Ag), anti-HCV antibody and ART were collected by a questionnaire or from medical records. Ten milliliters of ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood was collected from each participant. Plasma was then isolated from peripheral blood samples by centrifugation at 2,000 rpm for 10 min. In addition, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO, USA). RNA and DNA were then extracted from plasma and PBMC using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich), respectively.

Sero-epidemiology

Plasma samples were tested for anti-HIV antibodies using a commercially available rapid diagnostic kit [ABON HIV 1/2/O Triline Human Immunodeficiency Virus Rapid Test Devices; Abon Biopharm (Hangzhou) Co., Ltd., Hangzhou, China]. If the results of the rapid diagnostic kit were positive, HIV-1 genomic fragments were amplified by a polymerase chain reaction (PCR) to confirm the diagnosis of HIV-1 infection.

Amplification of HIV-1 Genomic Fragments

RNA and DNA were extracted from plasma and PBMC isolated from HIV-1-positive samples. Viral RNA in plasma RNA samples was reverse transcribed to cDNA using the SuperScript III First-Stand Synthesis kit (Invitrogen, Carlsbad, CA, USA) with the reverse primer, K-env-R1, 5'-CCAATCAGGGAAGAAGCCTTG-3' [corresponding to nucleotides (nt) 9168 to 9148 of the HIV-1 reference strain, HXB2 (GenBank accession no. K03455)]. The viral pol genes encoding protease (PR) (PR gene) and reverse transcriptase (RT) (RT gene) as well as partial fragments of the gag and env genes were then amplified from cDNA by a nested polymerase chain reaction (PCR) using Ex Taq polymerase (Takara Bio, Shiga, Japan) and the following primers. In the amplification of the PR gene, the primers for first PCR were DRPR05, 5'-AGACAGGYTAATTTTTTAGGGA-3' (nt 2074 to 2095) and DRPR02L, 5'-TATGGATTTTCAGGCCCAATTTTTGA-3' (nt 2716 to 2691), while the primers for nested PCR were DRPR01M, 5'-AGAGCCAACAGCCCCACCAG-3' (nt 2148 to 2167) and DRPR06,

5'-ACTTTTGGGCCATCCATTCC-3' (nt 2611 to 2592). In the amplification of the RT gene, the primers for first PCR were RT1L, 5'-ATGATAGGGGGAATTGGAGGTTT-3' (nt 2388 to 2410) and GPR2M, 5'-GGACTACAGTCY ACTTGTCCATG-3' (nt 4402 to 4380), while the primers for nested PCR were RT7L, 5'-GACCTACACCTGTCAACATAATTGG-3' (nt 2485 to 2509) and GPR3L, 5'-TTAAAATCACTARCCATTGYTCTCC-3' (nt 4309 to 4285). In the amplification of the gag gene encoding Gag p24, the primers for first PCR were H1G777, 5'- TCACCTAGAAC TTTGAATGCATGGG-3' (nt 1231 to 1255) and H1P202, 5'-CTAATACTGTATCATCTGCT GCTCCTGT-3' (nt 2352 to 2325), while the primers for nested PCR were H1Gag1584, 5'-AAAGATGGATAATCCTGGG-3' (nt 1577 to 1595) and G17, 5'-TCCACATTTC CAACAGCCCTTTTT-3' (nt 2040 to 2017). In the amplification of the C2-V3 regions of the env gene, the primers for first PCR were M5,-CCAATTCCCATACAT TATTGTGCCCCAGCTGG-3' (nt 6858 to 6889) and M10, 5'-CCAATTGTCCCT CATATCTCCTCCTCCAGG-3' (nt 7661 to 7632), while the primers for nested PCR were M3, 5'-GTCAGCACAGTAC AATGIACACATGG-3' (nt 6948 to 6973) and M8, 5'-TCCTTCCATGGGA GGGGCATACATTGC-3' (nt 7547 to 7521). PCR conditions are available upon request. If a viral gene fragment failed to be amplified from cDNA generated from plasma RNA samples even after multiple attempts, it was amplified instead from DNA extracted from PBMC samples. In order to examine the genomic fragment of the major viral population in a sample, PCR products amplified at the end-point dilution of DNA templates were subjected to a sequencing analysis.

Sequencing Analysis, HIV-1 Subtyping and the Detection of Drug Resistance-associated Mutations

A sequencing analysis of the amplified HIV-1 genomic fragment was performed using the BigDye Terminator v3.1 Cycle Sequencing kit with an ABI PRISM 3500 xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were then assembled and aligned using Genetyx version 10 software (Genetyx, Tokyo, Japan). HIV-1 subtyping was performed using the recombinant identification program (RIP), which is available on 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 MEGA6.06 software11 with

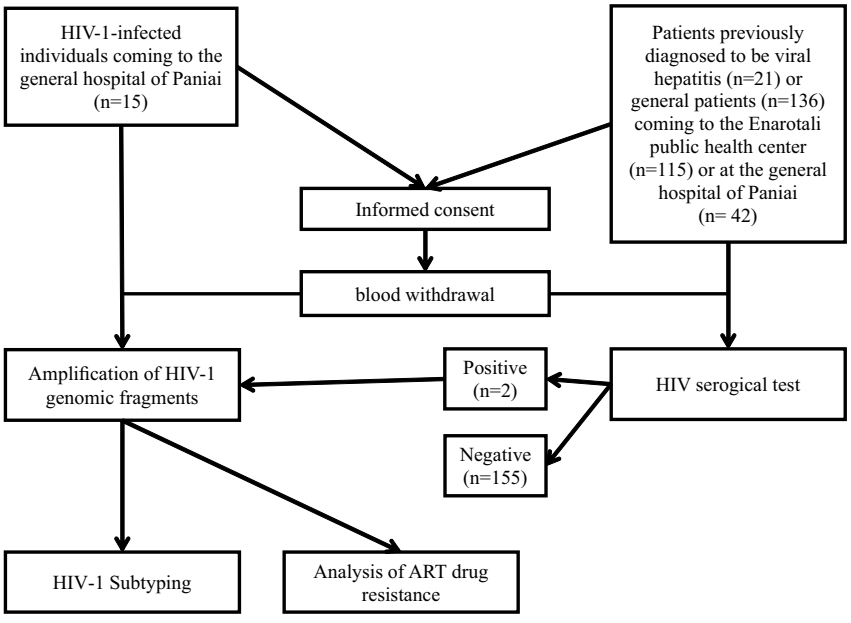

Figure 1. Flow chart of the sample collection and experimental plans

bootstrap values (1,000 replicates) for relevant nodes being reported on a representative tree. Viral subtyping was performed based on the successfully sequenced PR, RT, gag and/or env genes, and if there was an incompatibility in the subtype or CRF among the genes, the viral gene was considered to be from a recombinant form of HIV-1. In addition, the determination of drug resistance mutations in RT genes derived from individuals on ART was based on the guidelines published by the International AIDS Society United States (IAS-USA).[12] The nucleotide sequences of viral gene fragments have been deposited in the GenBank database under accession numbers KX639349-KX639352, KX639354-KX639358, KX639360-KX639368, KX639370-KX639387 and KX639389-KX639399.

RESULTS

Sero-epidemiological Study of HIV-1 in Papua Province

One hundred and fifty seven peripheral blood samples were corrected from the general population of Papua province in Indonesia. Forty-two and 115 samples were collected at the general hospital of Paniai and the Enarotali public health center, respectively (**Figure 1**). Serological tests revealed that 2 out of 157 samples (1.27%) were positive for anti-HIV antibodies. Two HIV-1-infected individuals were a 16-year-old male and a 36-year-old female, who were both married.

Demographic Information on Study Participants

In addition to 157 study participants, including 2 HIV-1-infected individuals, from the general population, 15 HIV-1-infected individuals were enrolled from the general hospital of Paniai for the genotypic analysis of HIV-1 (**Figure 1**). The demographic information of all study participants, consisting of 17 HIV-1-infected and 155 HIV-1-uninfected individuals, is shown in Table 1. Ten out of 17 (58.8%) HIV-1-infected individuals were female, and 3 out of 17 (17.6%) were HBs Ag-positive, indicating the co-infection of hepatitis B virus (HBV) and HIV-1. In contrast, none of the HIV-1-infected individuals were positive for the anti-hepatitis C virus (HCV) antibody, indicating no co-infection of HCV and HIV-1 among HIV-1-infected study participants. No significant differences were observed in the infection rates of HBV and HCV between HIV-1-infected and -uninfected individuals. The mean age of HIV-infected individuals was 25 years old, with the most predominant age groups being 20-29 and 30-39 years old. Fifteen out of 17 (88.2%) HIV-1-infected individuals were married (**Table 1**).

Table 1. Demographic information on HIV-positive and -negative study participants

	HIV (+)	HIV (-)
Gender (n)		
- Female	10	108
- Male	7	47
HBs Ag, n (%)		
- (+)	3 (17.64)	19 (12.25)
- (-)	14 (82.35)	136 (87.74)
Anti-HCV, n (%)		
- (+)	0 (0.0)	2 (129)
- (-)	17 (100.0)	153 (98.7)
Age (n)		
- <20 years	5	17
- 20-29 years	6	92
- 30-39 years	6	37
- ≥40 years	0	9
Marriage status (n)		
- Married	15	NA
- Unmarried	2	NA

HIV-1 Subtyping

Viral subtyping was performed using a phylogenetic tree analysis of successfully sequenced PR, RT, gag and env gene fragments (**Figure 2**). The results obtained showed that 8 out of 16 samples (50%) were classified as subtype B, six (37.5%) were classified as CRF01_AE, one sample (6.25%) was classified as subtype A1, and one sample (6.25%) was a recombinant form containing CRF01_AE and subtype B (CRF01_AE/B) genomic fragments (**Table 2**). In contrast, HIV-1 genomic fragments failed to be amplified from the sample, HIP 1 (**Table 2**). Viral subtyping was also performed using RIP, and the results obtained were consistent with that by a phylogenetic tree analysis (data not shown).

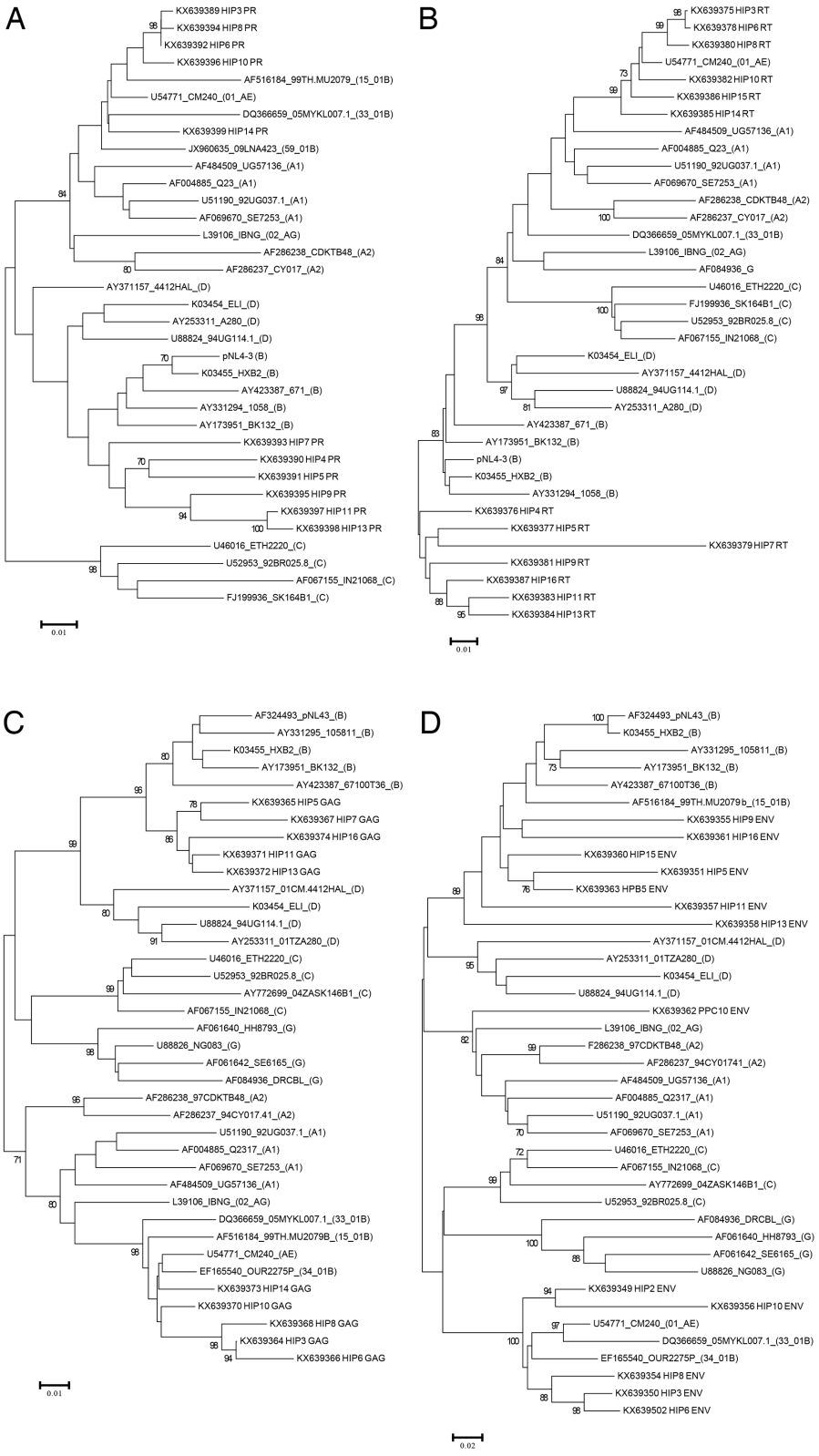

Figure 2. Phylogenetic analysis of HIV-1 PR, RT, gag and env gene sequences. Phylogenetic trees were generated for the newly sequenced HIV-1 PR (A), RT (B), gag (C) and env genes (D) together with the corresponding viral genes of reference HIV-1 strains representing subtype A1 (A1), subtype A2 (A2), subtype B (B), subtype C (C), subtype D (D), subtype G (G), CRF01_AE (01_AE), CRF02_AG (02_AG), CRF15_01B (15_01B), CRF33_01B (33_01B) and CRF34_01B (34_01B). The reference strains of the HIV-1 subtype are shown in bold. Sequence codes are presented as the GenBank accession number, patient ID or name of the reference strain, and the subtype or CRF of the reference strain (shown in parentheses) in order. Bootstrap values were shown when values were >70. CRF, circulating recombinant form.

Table 2. HIV-1 subtyping

Sample ID	subtype/CRF assignment	subtyping			
		RT gene	PR gene	env gene	gag gene
HIP 1	NA*	NA	NA	NA	NA
HIP 2	CRF01_AE	NA	NA	CRF01_AE	NA
HIP 3	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
HIP 4	B	B	B	NA	NA
HIP 5	B	B	B	B	B
HIP 6	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
HIP 7	B	B	B	NA	B
HIP 8	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
HIP 9	B	B	B	B	NA
HIP 10	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
HIP 11	B	B	B	B	B
HIP 13	B	B	B	B	B
HIP 14	CRF01_AE	CRF01_AE	CRF01_AE	NA	CRF01_AE
HIP 15	CRF01_AE/B**	CRF01_AE	NA	B	NA
HIP 16	B	B	NA	B	B
PPC10	A1	NA	NA	A1	NA
HPB 5	B	NA	NA	B	NA

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

HIV Drug Resistance-associated Mutations

Among 17 HIV-1-infected individuals, eight (47.1%) were on ART using RT inhibitors; therefore, the appearance of drug resistance-associated mutations was evaluated on RT genes derived from the samples of these individuals. The results obtained showed that three drug resistance-associated major mutations, K103N [amino acid substitution from lysine (K) to asparagine (N) at position 103 in RT], M184V and T215F, were detected in the viral RT gene derived from HIP 7. This patient used lamivudine (3TC), efavirenz (EFV) and tenofovir disoproxil fumarate (TDF) (**Table 3**). These drug resistance-associated major mutations were not detected in the other two patients who use same drug combinations (HIP 14 and HIP 16). No drug resistance-associated mutations were detected in the five remaining samples who used zidovudine (AZT), 3TC and nevirapine (NVP), or AZT, 3TC and EFV. In addition, no drug resistance-associated mutations were detected in patients who were not on ART.

In protease gene, no drug resistance-associated major mutations were detected (data not shown).

DISCUSSION

HIV/AIDS was initially detected in Indonesia in 1994. Since then, the epidemic has been steadily growing. The prevalence of HIV infection in Papua province (2.3%) is more than 5-fold among the general population in Indonesia (0.4%).[14] In addition, sexual behavior among the people of Papua was reported to increase the risk of HIV infection.[4] The HIV/AIDS situation in Papua province had been summarized in 2006[4]; however, recent information is limited. Two out of 157 healthy individuals from the general population were found to be infected with HIV-1. The prevalence of HIV was 1.27% in the present study, while it was previously reported to be 2.3%,[13] suggesting a decrease in its prevalence in the region. It is conceivable that large scale surveillance might be necessary for revealing the current prevalence of HIV in this region; however, difficulties are associated with conducting international collaborative studies there.

Demographic data on HIV-1-infected

Table 3. Clinical information of 17 HIV-infected individuals

ID	Anti-retroviral Treatment	Duration of HIV infection (month)	Types of ART	HBs Ag	Anti-HCV	Drug Resistance-associated Mutation in RT
HIP1	Yes	64	AZT + 3TC + NVP	-	-	-
HIP2	Yes	23	AZT + 3TC + NVP	-	-	-
HIP3	Yes	33	AZT + 3TC + NVP	-	-	-
HIP4	No	1		+	-	-
HIP5	No	0		-	-	-
HIP6	No	0		-	-	-
HIP7	Yes	34	3TC + EFV + TDF	-	-	K103N, M184V, T215F
HIP8	No	0		-	-	-
HIP9	No	0		-	-	-
HIP10	Yes	137	AZT + 3TC + EFV	-	-	-
HIP11	No	0		-	-	-
HIP13	No	0		+	-	-
HIP14	Yes	30	3TC + EFV + TDF	-	-	-
HIP15	Yes	31	AZT + 3TC +EFV	-	-	-
HIP16	Yes	5	3TC + EFV + TDF	+	-	-
PPC10	No	0		-	-	-
HBP5	No	0		-	-	-

Note: ART, antiretroviral therapy; 3TC, lamivudine; AZT, zidovudine; NVP, nevirapine; EFV, efavirenz; TDF, tenofavir

individuals showed that 15 out of 17 individuals (88.2%) were married. Consistent with the results of the present study, previous findings obtained in Jayapura, the provincial capital of Papua, showed that a large proportion of HIV-positive individuals were married (literature in Indonesian language). A significant proportion of men who have sex with men were married to women.[1,14] In addition, 25-60% of male injection drug users in Asia were either married or have an intimate partner.[14] Women are known to be at high risk of HIV infection from their husbands or intimate partners.[1] Therefore, it is important to conduct surveillance to reveal the conditions for HIV transmission between infected individuals and their partners in Papua province.

HIV-1 subtyping revealed that subtype B and CRF01_AE were the major subtype and CRF prevalent in Papua province in Indonesia. In addition, subtype A1 and a recombinant form containing CRF01_AE and subtype B were detected as minor strains. CRF01_AE is the predominant strain of HIV-1 in Southeast Asia[5]. It is also the predominant strain of HIV-1 in Indonesian cities such as Jakarta and Surabaya, while the CRF01_AE and subtype B recombinant forms have also been detected as minor strains in these regions.[6-9] In contrast, CRF01_AE and subtype B were prevalent as major strains in West Papua.[15,16] Consistent with the reports, subtype B and CRF01_AE were prevalent in Paniai, Papua as major strains. Since an unusual subtype in Southeast Asia, subtype A1 was also detected in this study, more complex recombinant forms and new CRFs might possibly be generated in the region in the future. Therefore, it is important to continue monitoring HIV-1 subtypes in Papua province. In addition, 3 drug resistance-associated major mutations, K103N, M184V and T215F, were detected from the RT gene derived from a patient, HIP 7. These are commonly occurring mutations against non-nucleoside reverse transcriptase inhibitors (NNRTIs) or nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). The patient had been on ART with a fixed-dose combination of TDF, 3TC and EFV. Same drug resistance-associated major mutations are also detected from a patient

who uses other first-line ART in Surabaya, Indonesia.[17] The appearance of drug resistance mutations against the first regime of ART as well as transmitted drug resistance need to be monitored in Papua province.

CONCLUSION

The current prevalence of HIV among the general population in Paniai, Papua is 1.27%, which is lower than that reported for Papua province in 2006. In addition, subtype B and CRF01_AE viruses were prevalent as major subtype and CRF in the region. Moreover, three drug resistance-associated major mutations were detected in one out of six samples. The limitation of this study is its low sample size, and intensified sero- and molecular epidemiological studies may be required in order to reveal the current situation of HIV infection in Papua province in Indonesia.

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AUTHORS' CONTRIBUTIONS

Masanori Kameoka and Nasronudin conceived and supervised the study; Muhammad Qushai Yunifiar M and Tomohiro Kotaki designed experiments; Muhammad Qushai Yunifiar M, Adiana Mutamsari Witaningrum, Siti Qamariyah Khairunisa and Dwi Wahyu Indriati performed experiments; Meilani and Tigor Yeheskiel collected samples and clinical information; Muhammad Qushai Yunifiar M, Tomohiro Kotaki, Adiana Mutamsari Witaningrum, Siti Qamariyah Khairunisa, Dwi Wahyu Indriati and Shuhei Ueda analysed data; Muhammad Qushai Yunifiar M wrote the manuscript; Tomohiro Kotaki, Siti Qamariyah Khairunisa, Shuhei Ueda and Masanori Kameoka made manuscript revisions.

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