Viral subtyping of HIV-1 derived from infected, drug-naive individuals in Jakarta, Indonesia

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Abstract. Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) (HIV/AIDS) remain a serious health problem in Indonesia. Jakarta has the largest number of individuals infected with HIV type 1 (HIV-1) in Indonesia; however, data on viral subtyping of HIV-1 is limited. We conducted a molecular epidemiological study on HIV-1 genes derived from infected, drug-naive individuals residing in Jakarta, the capital of Indonesia with large communities of HIV-infected individuals. To determine the distribution of HIV-1 subtypes circulating in Indonesia, 43 HIV-1-infected individuals were recruited in Jakarta. Gag and env genes were amplified from peripheral blood samples derived from study participants. Viral subtyping using the Recombinant Identification Program (RIP) and phylogenetic tree analysis were conducted. RIP and phylogenetic tree analysis revealed that CRF01_AE was the dominant subtype in Jakarta (40%), followed by recombinant viruses containing CRF01_AE and subtype B gene fragments (7%) and subtype B (7%). This study reported the emergence of CRF 01_AE as predominant subtype among HIV-1-infected, drug-naive individuals in Jakarta, Indonesia. Therefore, continuous surveillance is required in order to detect the emergence of HIV-1-infection.

1. Introduction
In Indonesia, there are 630000 individuals infected with human immunodeficiency virus (HIV) in 2015. Jakarta has the largest number of individuals infected with HIV type 1 (HIV-1) in Indonesia. In addition, the prevalence rate of HIV infection in Jakarta is highest among cities in Java island. HIV prevalence among adults (15-49 years old) reached 0.3% in 2015. HIV epidemic in Indonesia is among the fastest-growing in Asia [1].

HIV is divided into 2 types, HIV type 1 (HIV-1) and HIV type 2. HIV-1 can be further divided into four main groups, M (main), O (outlying), and N (new or non-M, non-O) and P (pending). The viruses
in group M are subdivided into many subtypes and circulating recombinant forms (CRFs). Subtypes A, B, C, D and G as well as CRF01_AE and CRF02_AG are considered to be major subtypes and CRFs and responsible for the worldwide pandemic of HIV-1. 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 [2] and is responsible for most infection cases in Indonesia [3]. In addition, several recombinants between CRF01_AE and subtype B, including CRF33_01B, have emerged in Indonesia [4,5]. Different subtypes and CRFs were considered to show different rates of disease progression, immune responses, responses to antiretroviral therapy and/or the development of drug resistance [6]; therefore, it is important to monitor the global prevalence of subtypes and CRFs for HIV prevention and control as well as for the vaccine development.

In order to clarify the viral subtypes of currently circulating HIV-1 strains in Jakarta, which has the highest HIV prevalence rate in Java island, we performed the genotypic characterization of viral genome derived from peripheral blood samples of HIV-1-infected, antiretroviral therapy (ART)-naïve individuals residing in Jakarta, Indonesia.

2. Methods

Forty-three HIV-1-infected, ART-naïve individuals were recruited from Soelianti Saroso Hospital, Jakarta. Ten milliliters of ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood samples were collected from participants between April 2014 and August 2014. Plasma was then isolated from peripheral blood samples by centrifugation for 10 min at 2,000 rpm. 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 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.

Viral of gag and env genes were amplified from DNA extracted from PBMC samples by a nested polymerase chain reaction (PCR) using Ex Taq (Takara Bio, Shiga, Japan) and the following primers. For the amplification of gag gene encoding Gag p24, the primers for the first PCR were H1G777, 5’-TCACTCTAGAACCTTTGAATGCATGGG-3’ (nt 777 to 801) and H1P202, 5’-CTAA TACTGTATCATCTGCTGCTCCTGT-3’ (nt 1874 to 1898), and the primers for the nested PCR were H1Gag1584, 5’-AAAGATGGATAACTCTGGG-3’ (nt 1123 to 1141) and G17, 5’-TCCACATTTCCAACAGGCCTTTTT-3’ (nt 1566 to 1589). For the amplification of env genes encoding the C2-V3 regions of gp120, the primers for the first PCR were M5, 5’-CCAAATCTCTCATATATTTGCGCCCCAGCTGG-3’ (nt 6858 to 6889) and M10, 5’-CCAATTGTTCTCATATCTTCTCCTCAGG-3’ (nt 7661 to 7632), and the primers for the nested PCR were M3, 5’-GTCAGCACTGCAATGTACATGACATGGG-3’ (nt 6948 to 6973) and M8, 5’-TCTTTGCATGAGGAGGGGACATGTT-3’ (nt 7547 to 7521). We had also tried to amplify viral gene fragments from plasma samples by reverse transcription-PCR essentially as described [7]; however, the amplification was failed probably due to an inadequate preservation condition of samples. The sequencing analysis of amplified viral gene fragments was carried out using the BigDye Terminator v3.1 Cycle Sequencing kit with an ABI PRISM 3500xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were then assembled and aligned using Genetyx version 10 software (Genetyx, Tokyo, Japan). The nucleotide sequences of gag and env genes have been deposited in the GenBank database under accession numbers MH595688-MH595717.

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 software [8] with Bootstrap values (1,000 replicates) for relevant nodes were reported on a representative tree. Viral subtyping was carried out based on the successfully sequenced gag and env genes, and if there is an incompatibility in the subtype or CRF among the gag and env genes, the viral gene was considered to be from a unique recombinant form (URF) of HIV-1. Viral subtyping by RIP was consistent with that by phylogenetic tree analysis.
3. Results
We collected 43 peripheral blood samples from HIV-1-infected, drug-naïve individuals residing in Jakarta. The partial fragments of HIV-1 *gag* and *env* genes were PCR-amplified and subjected to sequencing analysis. As the results, the sequencing analysis of 16 *gag* genes (460-bp; nt 863 to 1320) and 14 *env* genes (547-bp; nt 6975 to 7520) were successfully carried out. Viral subtyping revealed that CRF01_AE was the dominant subtype in Jakarta (40%), followed by recombinant viruses containing CRF01_AE and subtype B gene fragments (7%) and subtype B (7%). These results were consistent with a previous finding that CRF01 AE was the major CRF prevalent in Southeast Asia [5].

![Figure 1. Phylogenetic Tree Gag Gene of Patients HIV/AIDS](image-url)
4. Discussion
Our study revealed that CRF01_AE was the major CRF prevalent in Jakarta. In addition, recombinant viruses containing CRF01_AE and subtype B as well as subtype B viruses were found as minor HIV-1 strains. CRF01_AE is reported to be the predominant strain of HIV-1 in Southeast Asia. It is also the predominant strain in Indonesian cities such as Surabaya, Bali, Kepulauan Riau and Maumere [3,9-11], while CRF01_AE and subtype B were prevalent as major strains in West Papua [12]. In addition, recombinant viruses between CRF01_AE and subtype B have also been detected as minor strains in these regions [13]. Our results are relevant with the previous reports.

5. Conclusion
In summary, the genotypic study revealed that CRF01_AE were the major CRF prevalent in Jakarta. We consider that continuous surveillance to detect new strain possibly appear is required in the region.

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