Human T-lymphotropic Virus-1/2 detected in drug abused men who have sex with men infected with HIV in Surakarta, Indonesia

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Abstract. Human T-lymphotropic virus types 1 and 2 (HTLV-1/2) share similar routes of transmission with human immunodeficiency virus (HIV), and the HTLV-1/2 co-infection may affect the clinical course of HIV infection. The HIV/HTLV-1/2 co-infection risk higher if the patient performing the high-risk activities. This study evaluated the presentation of HTLV-1 and 2 in HIV-infected men who have sex with men with drug abused history in Surakarta Indonesia. Blood samples collected from HIV-infected men who have sex with men with drug abused history in Surakarta were tested using HTLV-1/2 enzyme-linked immunosorbent assays and confirmed by RT-PCR nested addressed the part of HTLV-1 LTR and HTLV-2 LTR region, respectively. The specificity of the molecular assays was confirmed by sequencing the amplicons. The anti HTLV-1/2 positive rate was 17.4% (8/46). All positive serological samples were confirmed by nested RT-PCR. Of these, three was HTLV-1 positive and five was HTLV-2 positive. Molecular analysis of positive PCR products revealed that all HTLV-1 isolates had a close relationship with HTLV-1 isolated in Japan while all HTLV-2 isolates with that of isolated in the USA. HTLV-1 and HTLV-2 were detected in drug abused men who have sex with men infected with HIV in Surakarta.

1. Introduction
Human T-cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus to be reported [1]. The retrovirus is endemic to certain regions of the world and infects around 10-20 million people [2]. The virus is associated with neoplastic, neurological, autoimmune, and infectious complications [1-3]. HTLV-2 is associated with a small increased risk of bacterial infections of the chest and bladder, increased cancer risk, and of HTLV- associated myelopathy/tropical spastic paraparesis [4,5].
HTLV-1/2 may affect the clinical course of human immunodeficiency virus-1 (HIV-1) and can lead to increased morbidity [6,7]. Moreover, HTLV-1/2 and HIV-1 mainly infecting CD4(+) T lymphocytes and also antigen-presenting cells such as dendritic cells (DCs) in a lesser extent [8]. Because HTLV-1/2 shares a similar transmission route with HIV, HTLV-1/2 infection may be more prevalent in HIV-infected individuals [6-7].

Drug abused history and high-risk sexual activities have been associated with HIV-1/HTLV-1 and HIV-1/HTLV-2 co-infections [1,2,7,9,10]. Previously, we reported the HTLV-1 and HTLV-2 were detected in the men who have sex with men with drug abused history in Surakarta, Indonesia [11]. Since the rates of HTLV-1/2 co-infection among drug abused men who have sex with men HIV-infected individuals have not been studied in Surakarta, we continued the study.

2. Materials and Methods
Since 2009, our research group (A-IGIC/A-Infection, Genomics, Immunology,& Cancer research group) has been conducting a molecular epidemiology study of human blood-borne viruses, by collecting epidemiological-clinical data and blood samples from the high-risk communities in Central Java, Indonesia, including that of the men who have sex with men with drug abused history in Surakarta [10-21]. Written informed consent was obtained from all the individuals who participated in the study. Approval was obtained from the institutional ethics committee review boards of the Faculty of Medicine of Universitas Sebelas Maret and the Dr. Moewardi General Hospital, Surakarta, Indonesia. Blood samples collected from HIV-infected men who have sex with men with drug abused history in Surakarta in 2010-2014 were used for the present study (n=46).

2.1. Immunological Assay
Blood samples aliquot obtained were subjected to the immunological assays as for the presence of specific antibodies to HTLV-1/2 using an ELISA kit that employs HTLV-1/2 viral lysates plus recombinant proteins from HTLV-1/2 as antigens (HTLV-I/II ELISA 4.0, MP Biomedicals, Santa Ana, California). All assays were performed according to manufacturer’s instructions in duplicate [10-13].

2.2. Nucleic Acid Extraction and HTLV-1/2 Molecular Detection
All blood samples were confirmed by a molecular assay as previously described [10-13]. Viral nucleic acid was extracted from plasma by using High Pure Viral Nucleic Acid Kit (Roche Life Science, Mannheim, Germany) according to the manufacturer’s instructions. DNA was extracted from whole blood by using High Pure PCR Template Preparation Kit (Roche Life Science). The nucleic acids were then aliquoted, and one aliquot was reverse-transcribed according to the Transcriptor High Fidelity cDNA Synthesis Kit protocol using random hexamers (Roche Life Science). Molecular detection was performed by nested PCR using the FastStart High Fidelity PCR System (Roche Life Science). LTR segments of HTLV-1 and HTLV-2 were amplified to confirm HTLV-1 and HTLV-2 infection. Briefly, the segment of LTR regions of the HTLV-1 genome was amplified using primers LTR1/LTR3 in the first round and LTR1/LTR2 in the second round while the segment of LTR regions of the HTLV-2 genome was amplified using primers VS1/VS2 in the first round and VS3/VS4 in the second round. Internal amplification controls were included to exclude any false-negative results. Corresponding positive controls and one negative control (sterile water) were included in each group. The specificity was confirmed by sequencing the amplicons. All samples were tested at least in duplicate.

2.3. Sequencing and Phylogenetic Analysis
Determination of nucleotide sequences and phylogenetic analysis were performed as described previously [10-13]. Briefly, the PCR products were purified from agarose gells and then subjected to the determination of nucleotide sequences directly with the primers of LTR1/LTR2 for HTLV-1 LTR region and VS3/VS4 for HTLV-2 LTR region, respectively. Initial genotyping was conducted using
the NCBI genotyping tool. Sequences were then submitted to the BLAST program in order to check their similarity to related strains deposited in Genbank/EMBL/DDJB. The reference strain with the highest homology score to each analyzed strain was retrieved from the GenBank/EMBL/DDJB database and then was aligned with the tested sequences. Kimura two-parameter method was used to estimate the frequency of nucleotide substitution at each base. A phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap replications to estimate its reliability. The phylogenetic tree was constructed using the CLC Main Workbench 7.9.1 software package (CLC Bio).

3. Results and Discussion

Out of 46 HIV-positive blood samples, eight (17.4%) were positive for anti-HTLV-1/2 antibodies. Nested PCR was possible to be performed in all positive samples, confirmed the presence of DNA/HTLV in the plasma and whole blood for the LTR regions of the genome. Of these, three was HTLV-1 positive and five was HTLV-2 positive. Based on 317 nucleotides part of HTLV-1 LTR region, the all HTLV-1 isolates was shared 100 % homology with B1033-2009 (HTLV-1 isolated in Japan; GenBank Accession Number AB513134) and 09IDSKAH-1-3 (HTLV-1 isolated in Central Java, Indonesia, GenBank Accession Number JN247457), consistent with previous results [10-11]. Based on 666 nucleotides part of HTLV-2 LTR region, all HTLV-2 isolates had shared 99 % homology with HTLV-2 isolated in USA (GenBank Accession Number AF412314) and HTLV-2 isolated from Central Java, Indonesia (09IDSKAH-2-1, GenBank Accession Number JN247458), consistent with previous results [10-11].

HTLV-1/HIV co-infection is known to elevate the CD4+ T-cell counts of treatment-naïve persons. Also, HTLV-1/HIV co-infected participants continued to have elevated CD4+ T-cell counts after developing virologic failure on ART, despite no difference in their HIV viral load levels when compared with HIV mono-infected participants; therefore, the CD4+ T-cell count testing maybe not be a useful strategy to monitor ART response in the presence of HTLV-1 infection [22]. The HTLV/HIV co-infection was detected in 17.4% of drug abused men who have sex with men infected with HIV in Surakarta, indicated the necessity of HTLV detection in HIV patients with high-risk histories of respected co-infection. Moreover, co-infection with HTLV-1 has been associated with faster progression to AIDS [23]. HTLV-1 infection can modify the expression of main functional transcription factors, FOXP3 and GITR, which may lead to immune response deterioration of Tregs [24].

HTLV viral transmission requires cell-to-cell contacts, while cell-free virions are poorly infectious and almost absent from body fluids; therefore, horizontal transmission between adults is likely the main route of HTLV infection in the general population and that this is likely to occur through sexual contact and or sharing blood contain injecting needles as performed by the injecting drug abused users [25,26]. Health intervention like increasing availability of free condoms methadone therapy provided by the government may decline the HTLV seroprevalence [27]. Moreover, other co-infection status study should be performed in the community, as previously [28-33].

4. Conclusions

The HTLV-1 and HTLV-2 were detected in the HIV-infected men who have sex with men with drug abused history in Surakarta, Indonesia.

5. References

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