Molecular status of Human Immunodeficiency Virus, Hepatitis B virus, and Hepatitis C virus among injecting drug male commercial sex workers in Surakarta, Indonesia

Afiono Agung Prasetyo1,2,3*, Marwoto1,2,3, Zainal Arifin Adnan1,4 and Hartono1,5

1 A-IGIC (A-Infection, Genomic, Immunology,& Cancer) Research Group, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia.
2 Center of Biotechnology and Biodiversity Research and Development, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia.
3 Division of Virology, Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia.
4 Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia.
5 Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia.

*Corresponding author: afie.agp.la@gmail.com; afieagp@yahoo.com; afie@staff.uns.ac.id

Abstract. Male commercial sex workers are one of the high-risk community for blood-borne viruses. However, there are no data concerning the molecular status of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) circulated among male commercial sex workers with injecting drug habits in Surakarta, Indonesia. Blood samples obtained from injecting drug male commercial sex workers in Surakarta were examined for HIV antibodies, HBsAg, and HCV antibodies, respectively, by immunological assays. Blood samples were also subjected to viral nucleic acid extraction and molecular detection of HIV, HBV, and HCV by nested (RT) PCRs. The PCR products were purified from agarose gels, and the nucleotide sequences were retrieved and molecular analyzed. HIV, HBV, and HCV were detected in 29.4% (10/34), 17.6% (6/34), and 52.9% (18/34), respectively. HIV CRF01_AE and B were found to be circulating in the community. HBV genotype B3 was predominated, followed by C1. HCV genotype 1a was predominated, followed by 1c, 3a, 1b, and 4a. HIV, HBV, and HCV were found circulating in the male commercial sex workers with injecting drug habits in Surakarta, Indonesia.

1. Introduction
Male commercial sex workers still considered not accepted in Indonesian society, therefore become a neglected population in the community. In a fact, male commercial sex workers represent a key population in Human Immunodeficiency Virus (HIV) epidemic [1]. Injecting drug abused history is a risk factor for blood-borne virus infection; therefore, male commercial sex workers with injecting drug abused history will have a higher risk of blood-borne virus infection, including HIV infection, also for Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) [2]. Previously, we reported the presence of
HIV, HBV, and HCV in male commercial sex workers in Surakarta [3-4]. We then continued the study, to evaluate the circulation of HIV, HBV, and HCV in male commercial sex workers with injecting drug abused history in Surakarta, Indonesia.

2. Materials and Methods

2.1. Study Population
Since 2009, our research group (A-IGIC/A-Infection, Genomic, 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 male commercial sex workers in Surakarta [3-4]. A 5-ml blood sample was collected from willing participants and then fractionated, aliquoted, and stocked in -80 °C until further study. 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. All blood samples collected from male commercial sex workers with injecting drug abused history in Surakarta in 2010-2014 were used for the present study (n= 34).

2.2. Immunological Assays
Blood samples aliquot obtained were subjected to the immunological assays as described previously [3-6]. Briefly, HIV antibodies were detected using a Determine HIV-1/2 Kit (Abbott Diagnostics Japan, Tokyo, Japan) and positive results were confirmed using Vironostika HIV Uniform II Antigen Ag/Ab (BioM’erieux, Marcy l’Etoile, France). A SERATEC Hepatitis B Quick Test (Gesellschaftfür Biotechnologie GmbH, Göttingen, Germany) and Ortho HCV PA II (Ortho Diagnostics, Tokyo, Japan) were used for the detection of the HBsAg and anti-HCV, respectively. All assays were performed according to manufacturer’s instructions in duplicate.

2.3. Nucleic Acid Extraction and Molecular Detection
The viral nucleic acid extraction and molecular detection techniques were performed as described previously [2,5,8]. Briefly, a portion of the HIV gag gene encoding the p24 region was amplified by nested RT-PCR. A portion of the HBV HBsAg gene was amplified by nested PCR. A portion of the E1–E2 region of the HCV genome were amplified by nested RT-PCR. 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.

2.4. Sequencing and Phylogenetic Analysis
Determination of nucleotide sequences and phylogenetic analysis were performed as described previously [2,8]. Briefly, the PCR products were purified from agarose gels, and the nucleotide sequences were determined for the HIV gag region (position 1577-2039 on HIV-1 reference HXB2), HBV HBsAg region (corresponding to 255-671 nt in HBV ADW GenBank Accession Number V00866), and HCV E1-E2 region (position 1322-1859 in H77 strain, GenBank Accession Number NC_004102), respectively. Initial genotyping was conducted using the NCBI genotyping tool. The sequences were then submitted to the Blast program to check their similarity to related strains deposited in Genbank/EMBL/DDBJ. The reference strains with the highest homology score to each analyzed strain were retrieved from the GenBank/EMBL/DDJB databases and aligned with the tested sequences. All the HIV, HBV, and HCV sequences isolated in Indonesia deposited in GenBank were also included in the alignment analysis for each tested sequence. The frequency of nucleotide substitution at each base was estimated by the Kimura two-parameter method. A phylogenetic tree was constructed by the neighbor-joining method, and its reliability was estimated by 1000 bootstrap replications. The phylogenetic tree was constructed using the CLC Main Workbench 7.9.1 software package (CLC Bio).
3. Results and Discussion
This is the first molecular epidemiology study of HIV, HBV, and HCV in male commercial sex workers with injecting drug abused history in Surakarta, Indonesia. For the best of our knowledge, prior to this study, there were only limited data about molecular epidemiology profiles of the respected viruses from the community in Indonesia, especially from Surakarta.

All blood samples obtained from injecting drug male commercial sex workers in Surakarta subjected for the HIV, HBV, and HCV immunological assays. Consistent with previous reports [2,3,8], the anti-HIV-1/2, HBsAg, and anti-HCV were found in 29.4% (10/34), 17.6% (6/34), and 52.9% (18/34) samples, respectively. All blood samples were also subjected to molecular assays. HIV, HBV, and HCV were detected in 29.4% (10/34), 17.6% (6/34), and 52.9% (18/34) samples, respectively. None of the respected genome viruses were detected from the negative immunological assays plasma samples.

A portion of the HIV gag gene could be amplified by nested RT-PCR in ten anti-HIV samples. The amplified portions of the HIV-1 gag region of ten HIV isolates (HIDSKA-31, HIDSKA-32, HIDSKA-33, HIDSKA-34, HIDSKA-35, HIDSKA-36, HIDSKA-37, HIDSKA-38, HIDSKA-39, and HIDSKA-40) were genotyped. The six HIV isolates were clustered together in the phylogenetic tree and shared 96-98% nucleotide homology with HIV-1 CRF01_AE isolated in drug abused inmates in Central Java (09IDSKA-18). The four HIV isolates were genotyped as B, shared 98% homology with HIV-1 B isolate HIVHXB2CG [9] (figure 1). The results obtained were consistent with HIV circulated in drug abused inmates in Central Java’s correctional facilities [2] and transgender commercial sex workers in Surakarta [8].

![Figure 1](image)

**Figure 1.** Phylogenetic Analysis of HIV Isolates Obtained from The Male Commercial Sex Workers with Injecting Drug Abused History in Surakarta Based on HIV-Gag Nucleotide Sequences

A portion of the HBV preS2/S gene was successfully amplified in six HBsAg positive samples. The HBIDSKA-21, HBIDSKA-22, HBIDSKA-23, and HBIDSKA-24 were clustered together with HBV genotype B3 isolated in Central Java (09IDSKAB-5). The HBIDSKA-25 and HBIDSKA-26 were closely related to HBV genotype C1 isolated in Central Java (09IDSKAB-14) [2] (figure 2). The results obtained were consistent with that of in transgender commercial sex workers in Surakarta, as reported previously [8]. Moreover, the HBV genotypes in the present report were already reported found circulated in drug abused inmates in Central Java’s correctional facilities [2].
Figure 2. Phylogenetic Analysis of HBV Isolates Obtained from The Male Commercial Sex Workers with Injecting Drug Abused History in Surakarta Based on HBV HbsAg Nucleotide Sequences

All blood samples positive for anti-HCV were found positive for HCV RNA by nested RT-PCR (n= 18). The PCR products were then subjected to the determination of the E1-E2 sequence. HCV genotypes 1a was found in 8 subjects (HCIDSKA-45, HCIDSKA-46, HCIDSKA-47, HCIDSKA-48, HCIDSKA-49, HCIDSKA-50, HCIDSKA-51, and HCIDSKA-52) and were clustered together with HCV 1a isolated in Central Java (09IDSKAC-18) [2]. HCV genotypes 1c was found in four subjects (HCIDSKA-53, HCIDSKA-54, HCIDSKA-55, and HCIDSKAC-56) and were clustered together with HCV 1c isolated in Central Java (09IDSKAC-7) [2]. HCV genotypes 3a was found in three subjects (HCIDSKA-57, HCIDSKA-58, and HCIDSKA-59) and were clustered together with HCV 3a isolated in Central Java (09IDSKAC-2) [2]. HCV genotype 1b was found in two subjects (HCIDSKA-60 and HCIDSKA-61) were clustered together with HCV 1b isolated in Central Java [2]. HCV genotype 4a was found in 1 subject (HCIDSKA-62) and was clustered together with HCV 4a isolated in Central Java (09IDSKAC-13) [2]. The present data indicated all HCV circulated in male commercial sex workers with injecting drug abused history in Surakarta were consistent with HCV circulated in drug abused inmates in Central Java’s correctional facilities.

Previously, we proposed that drug abuse prisoner is useful as a surrogate sentinel to gain molecular epidemiology data on human blood-borne viruses for a country such as Indonesia in which it is difficult to perform active surveillance among the general population [2]. In the present study, the molecular data of HIV, HBV, and HCV found in the male commercial sex workers with injecting drug abused history in Surakarta were consistent with the respected viruses in drug abuse prisoner imprisoned in Central Java’s correctional facilities. In general, the male commercial sex workers with injecting drug abused history in Surakarta were found at high risk for blood-borne viruses infection, consistent with previous reports [2,8,10-12]. People engaging in transactional sex are considered a key population for HIV prevention; therefore, health interventions are needed to improve knowledge, risk perception, and health behaviors in the key population [13]. Moreover, co-infection and polymorphisms status study should be performed for the community, and the pathogen found should be molecularly characterized as previously [14-22].

4. Conclusions
HIV, HBV, and HCV were found circulating in the male commercial sex workers with injecting drug abused history in Surakarta, Indonesia.

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