Jpn. J. Infect. Dis., 69, 493–499, 2016

Original Article

High Rates of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Human Immunodeficiency Virus Infections and Uncommon HBV Genotype/Subtype and HCV Subtype Distributions among Transgender Individuals in Surabaya, Indonesia

Alfonsus Adrian Hadikusumo[1], Takako Utsumi[2,3]*, Mochamad Amin[2], Siti Qamariyah Khairunisa[2], Anittaqwa Istimagfirah[2], Rury Mega Wahyuni[2], Maria Inge Lusida[1,2], Soetjipto[1,2], Edhi Rianto[1], Juniastuti[1,2], and Yoshitake Hayashi[3]

[1]*Faculty of Medicine, Airlangga University, Surabaya;*
[2]*Indonesia-Japan Collaborative Research Centre for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia; and*
[3]*Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan*

SUMMARY: Transgender people are at a high risk for sexually transmitted viruses such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Moreover, Indonesia has a moderate-to-high rate of HBV infection and rapid epidemic growth of HIV infection; hepatitis C virus (HCV) infection can co-occur with HBV and HIV infections. In this study, 10 of 107 individuals (9.3%) were positive for HBV surface antigen (HBsAg) and/or HBV DNA, whereas 19 of 101 individuals (18.8%) with negative results for HBsAg were positive for HBV core antibody (anti-HBc). Seven of the 107 individuals (6.5%) were anti-HCV positive, and 16 of the 100 tested samples (16.0%) were HIV positive. Genotype and subtype analyses of all 10 HBV DNA (6 HBsAg positive and 4 anti-HBc positive) strains showed that 3 were of the HBV genotype/HBsAg subtype C/*adrq*$^+$, one was of C/*adw2*, and 5 were of B/*adw2*. The HCV subtype distribution showed that 33.3% were of HCV-1b, and 66.7% were of HCV-3k (n = 6). These distributions differed from those found in the general population of Surabaya, Indonesia. Interestingly, HIV subtype analysis showed a high prevalence of HIV, with possible recombinants of CRF01_AE and subtype B.

INTRODUCTION

Transgender people commonly have risky sexual behaviors and are at an increased risk of for sexually transmitted viruses (1). Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infection can lead to severe chronic infections (2). Because hepatitis C virus (HCV) infection can co-occur with HBV and HIV infections, it might also be sexually transmitted (3), especially among people with multiple sexual partners, but to a much lesser extent than HBV infection (2).

Indonesia is a country with a moderate-to-high (6–9%) rate of HBV infection (4). According to the report from the United Nations Program on HIV/AIDS (UNAIDS), Indonesia has shown a rapid epidemic growth of HIV, and the estimated number of people with HIV has increased approximately 32-fold from 12,000 in 2001 to 380,000 in 2011 (5,6). The prevalence of HCV in the general population during this period was 2.1–2.3% in Indonesia (7,8).

Received August 6, 2015. Accepted February 8, 2016.
J-STAGE Advance Publication March 18, 2016.
DOI: 10.7883/yoken.JJID.2015.384
*Corresponding author: Mailing address: Center for Infectious Diseases, Graduate School of Medicine, Kobe University, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan. Tel: +81-78-382-5700, Fax: +81-78-382-5719, E-mail: tutsumi@people.kobe-u.ac.jp

HBV and HCV genotypes show marked geographic and ethnic distributions. HBV isolates of different genotypes and subtypes show different geographical distributions, virological characteristics, and possibly, clinical outcomes (9,10). Genotypes B and C (HBV/B and HBV/C, respectively) are predominant in Asia and particularly, in Indonesia (11,12). Indonesia is a multiethnic country. We previously reported the distribution of the genotypes and subtypes of HBV across several geographical areas in Indonesia (9,13). In addition, we reported the prevalence of each HCV subtype, including HCV subtype 1c (HCV-1c), among various clinical populations in Indonesia.

Because the data regarding the genomic characterization of HIV, HBV, and HCV are scarce with regard to the global transgender population (1,14), this study aimed to characterize the HBV, HCV, and HIV subtypes circulating among this community in Surabaya, Indonesia, at the molecular level.

MATERIALS AND METHODS

Serum samples: A total of 107 serum samples were collected from a group of transgender individuals under supervision at a health center in the port area in Surabaya, Indonesia, in 2012. The personnel at the health center first determined whether the individuals were transgender from their appearance and interview. Thereafter, the individuals were asked to join the community supervised by the health center in order to

receive regular follow-up to detect sexual transmitted diseases. In this study, a questionnaire was used to obtain information on the identity, orientation, and partners, as well as a history of previous infection. All individuals enrolled (100%) were transgender women who were born as men but later identified themselves as women. They did not have a history of previous HBV, HCV, or HIV infection. Before examination, all of the sera were stored at −80°C at the Institute of Tropical Disease, Airlangga University. Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine Airlangga University and Kobe University, Japan. All the participants signed an informed consent document and volunteered for this study.

HBV serological tests, DNA extraction, and PCR amplification: The serum samples were screened for HBsAg using a reverse passive hemagglutination assay (Mycell II HBsAg; Institute of Immunology, Tokyo, Japan) and anti-HBc using passive hemagglutination (Mycell II anti-rHBc; Institute of Immunology).

A part of the S-gene from the samples that tested positive for HBsAg or anti-HBc was amplified using PCR for HBV DNA detection. HBV DNA was extracted from 200 μL serum using a DNA extractor kit (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). The presence of HBV DNA was assayed using PCR with P7 (5′-GTGGTGGACTTCTCTCAATTTTC-3′, nucleotides [nt] 256–278) and P8 (5′-CGGTAWAAAGGGACTCAMGAT-3′, nt 796–776) primer pairs to detect the presence of the S-gene (nt 256–796). When PCR amplification results were negative, a second-round (nested) PCR was conducted using primers HBS1 (5′-CAAGGTATGTTGCCCGTTTG-3′, nt 455–474) and HBS2 (5′-AAAGCCCTGCGAACCACTGA-3′, nt 713–694) (9). Both first- and second-round PCRs consisted of a 5-min denaturation at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C (15).

HCV serological test, RNA extraction, and reverse transcription (RT)-PCR amplification: The presence of anti-HCV antibody in the sera was detected using the reverse particle hemagglutination method (Ortho HCV Ab PA test II; Ortho-Clinical Diagnostics, Tokyo, Japan).

Serum samples that tested positive for the anti-HCV antibody were further analyzed for the presence of HCV RNA. HCV RNA was extracted from 140 μL of serum using a commercially available kit (QIAamp Viral RNA kit; Qiagen).

To amplify the NS5B region of the HCV genome, the extracted RNA was reverse-transcribed and amplified using SuperScript One-Step RT-PCR (Invitrogen, Carlsbad, CA, USA). PCR was performed using Platinum Taq DNA polymerase (Invitrogen). The reaction was initially performed at 45°C for 30 min for RT and at 94°C for 2 min, followed by the first-round of PCR for over 40 cycles. Each cycle consisted of a pre-denaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 56°C for 40 s, and an extension at 72°C for 1 min using the outer primers F1 (5′-CAATWSMMACBACCATCATGGC-3′, nt 7999–8020) and R1 (5′-CAGGARTTRACTGGAGTGTG-3′, nt 8824–8805). The second round of PCRs was performed under the same conditions used to amplify the HCV genome using the inner primers F2 (5′-ATGGGHHSBKCMTAYGGATTCC-3′, nt 8160-8181) and R2 (5′-CATAGCNTCCGTGAANGCTC-3′, nt 8630–8611) (16). All PCRs were performed using Hot Star Master Mix (Qiagen).

Anti-HIV antibody test and HIV type 1 (HIV-1) genomic fragment amplifications: One hundred serum samples were screened for HIV using 3 methods: a rapid diagnostic test (HIV 1/2 one step rapid test; Zhejiang Orient Gene Biotech, Zhejiang, China) followed by a double antigen/antibody sandwich enzyme linked immunoassay (EIA; HIV 1/2/O Antigen/Antibody; ACON Laboratories, San Diego, USA) and an immuno-chromatographic assay system (MONO 1/2 HIV Test; Shanghai Huaguan Biochip, Shanghai, China).

The samples that were positive for anti-HIV antibody were amplified to obtain their proviral DNAs. They were extracted from peripheral blood mononuclear cells (PBMC) using QIAamp DNA Blood Mini Kit. For the amplification of the viral *pol* gene fragment, UNIPOL 5 (5′-TGGGTACCAGCACACAAAGGAATAGGAGGAAA-3′, nt 4152–4183) and UNIPOL 6 (5′-CCACAGCTGATCTCTGCCTTCTCTGTAATAGA-CC-3′, nt 4934–4901) were used for the first PCR, while UNIPOL 1 (5′-AGTGGATTCATAGAAGCAGAAGT-3′, nt 4470–4492) and UNIPOL 2 (5′-CCCCTATTCCTCCCCTTCTTTTAAAA-3′, nt 4806–4781) were used for nested PCR. The first PCR of *pol* gene consisted of one cycle of denaturation for 5 min at 94°C; 35 cycles of 1 min at 94°C for denaturation, 1 min at 45°C for annealing, and 1 min at 72°C for extension; and a final extension cycle of 5 min at 72°C. For the second PCR for *pol* gene amplification, the annealing temperatures were changed to 50°C, 55°C, and 60°C, respectively (6). In addition, for amplification of the viral *gag* gene fragment, H1G777 (5′-TCACCTAGAACTTTGAATGCATGGG-3′) and H1P202 (5′-CTAATACTGTATCATCTGCTCCTGT-3′) were used for the first PCR, while H1gag1584 (5′-AAAGATGGATAATCCTGGG-3′) and g17 (5′-TCCACATTTCCAACAGCCCTTTTT-3′) were used for nested PCR. The PCR conditions were as follows. For the first PCR of *gag* gene amplification, one cycle of 3 min at 95°C for denaturation; 35 cycles of 1 min at 95°C for denaturation, 1 min at 58°C for annealing, and 1 min at 72°C for extension; and a final extension cycle of 3 min at 72°C were carried out. For the nested PCR of *pol* gene amplification, an annealing temperatures of 49.4°C was used.

Sequence and phylogenetic analysis: The nucleotide sequences of the amplified fragments of HBV, HCV, and HIV were determined using the BigDye Terminator v3.1 Cycle Sequencing kit with an Applied Biosystems 3500xL Genetic Analyzer (Foster City, CA, USA) (9,17,18).The sequences were compared with those from the international DNA databank (DDBJ/EMBL/GenBank).

The HBV subtypes were deduced based on the predicted amino acid sequences of HBsAg (12,19). HBV subgenotypes were determined based on homologies (>96%) in the S gene using Genetyx-Win v10.0 (Genetyx, Tokyo, Japan) and phylogenetic analysis.

HCV subtypes were determined by homologies in the NS5B region. When a subtype assignment was could not be completed because of the lack of NS5B amplification, the nt sequences of the 5′ untranslated region were determined and compared with the consensus sequence motifs for each of the major genotypes reported previously (20,21). When the sequence of an HCV clone completely matched the consensus motifs of a major genotype, the HCV clone was assigned to a genotype (e.g., HCV type 1) (22).

HIV-1 subtyping was conducted using the Recombination Identification Program (RIP) available at the website of the HIV sequence database <http://www.hiv.lanl.gov/>.

The phylogenetic analysis of HBV, HCV, and HIV-1 was conducted using neighbor-joining trees with the Kimura two parameter model, which were constructed using MEGA6.2 software, after multiple alignment using the Clustal W algorithm and manual editing (23,24).

RESULTS

Samples: Peripheral blood samples were collected from 107 transgender individuals (mean age, 33.4 years; age range, 19–60 years), including 81 Javanese people, 16 Maduranese people, 7 people of other ethnicities (the descent of these ethnicities was carefully documented for 3 previous generations, both paternally and maternally), and 7 people of unknown ethnicity. All samples were collected from transgender people who had male partners. None of the participants had a previous history of intravenous drug user or any other blood-transmitted contact. These data were acquired from questionnaires and interviews. The primary risk factor for infection among these individuals was sexual intercourse.

HBV infection among transgender individuals in Indonesia: The sero-epidemiological tests revealed that 6 of the 107 individuals (5.6%) were HBsAg positive. In addition, 19 of the 101 individuals (18.8%) who were HBsAg negative were also anti-HBc positive (Table 1). In this study, we defined HBV infection as HBsAg positivity and/or HBV DNA positivity. Ten HBV DNA-positive samples (9.3%) were detected, including 6 HBsAg-positive samples and 4 HBsAg-negative samples with anti-HBc positivity. Based on the homology of the reported isolates in the DDBJ/EMBL/GenBank, 6 samples were of genotype B; interestingly, however, the other 4 samples were of genotype C, which is an uncommon in Surabaya. Nine samples were analyzed to determine the subtypes. Five of the 9 isolates belonged to the genotype/subtype B/*adw2*, one belonged to C/*adw2*, and 3 belonged to C/*adrq*$^+$ (Fig. 1 and Table 2). In addition, one sample could not be analyzed because the sequence was not long enough.

Regarding the subgenotype of the HBV, the genotype C isolates (4/4) formed a separate cluster from those including subgenotypes C1 to C16. Isolates with the genotype B belonged to the subgenotype B3. Two samples could not be analyzed because their sequences were not long enough (Fig. 2).

Table 1. Prevalence of HBsAg, anti-HBc, anti-HCV, and anti-HIV antibodies among transgender people in Indonesia

Virus	Serological test	No. positive/ No. tested (%)	No. positive HBV DNA or HCV RNA/No. tested
HBV	HBsAg	6/107 (5.6)	6/6[1)]
	anti-HBc	19/101 (18.8)[2)]	4/19[1)]
HCV	anti-HCV	7/107 (6.5)	7/7
HIV	anti-HIV	16/100 (16.0)	–

[1)]: A total of 10 HBV DNA-positive samples were detected among transgender individuals with positivity of HBsAg or anti-HBc.
[2)]: A total of 101 samples those were negative for HBsAg were tested for anti-HBc.

Table 2. Prevalence of HBV genotypes/subtypes and HCV subtypes among transgender individuals in Indonesia

Virus (total sample)	Type	*n*
HBV (10[1)])	B/*adw2*	5
	C/*adrq*$^+$	3
	C/*adw2*	1
HCV (7[2)])	HCV-1b	2
	HCV-3k	4

[1)]: One HBV DNA sample was not suitable for further analysis.
[2)]: One HCV RNA sample was not long enough to be analyzed. No person was co-infected with HBV and HCV.

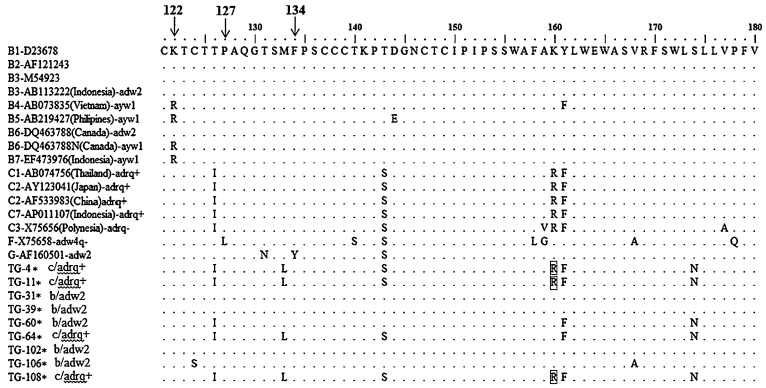

Fig. 1. Multiple alignment of amino acid sequences of HBsAg (positions 121–180) of HBV isolates from transgender people in Indonesia (code TG; shown with asterisk) and those from international DNA data bank (indicated with the accession numbers and countries of origin; genotypes, subgenotypes, and subtypes are also indicated).

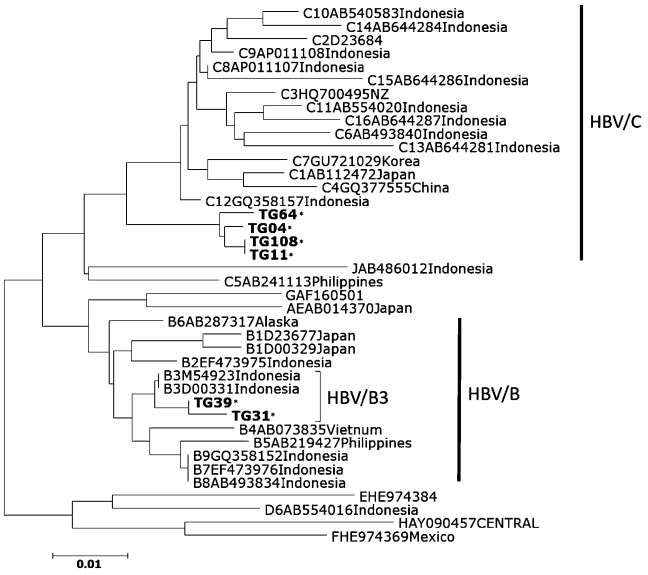

Fig. 2. Phylogenetic analysis of HBV isolates from transgender people in Indonesia (shown in bold letter with asterisk) and those from international DNA data bank (indicated with the subgenotype and accession number), on the basis of the S region.

HCV infection among transgender individuals: Seven of the 107 individuals (6.5%) were anti-HCV positive (Table 1). HCV RNA was detected in all anti-HCV positive samples. The analysis of the 6 HCV RNA isolates showed that 2 were of subtype 1b, and the other 4 were of subtype 3k (Fig. 3 and Table 2). One sample could not be analyzed because the sequence was not long enough.

HIV infection among transgender individuals: Sixteen of the 100 samples tested (16.0%) were anti-HIV positive (Table 1). Fifteen of the 16 samples were successfully amplified and were subject to sequence analysis. Eleven of the 16 samples (Fig. 4) were positive in the *pol* region, and the other 12 samples were positive in the *gag* region. The viral subtype acquired from the RIP program showed that all *pol* (11/11, 100%) and 8 of 12 *gag* (66.7%) (Fig. 4) gene fragments were of subtype CRF01_AE, whereas the other 4 (33.3%) *gag* gene fragments turned out to be of subtype B (strains no. GAG TG 101, 105, 106, and 111). There were 2 possible recombinant strains (GAG TG 106 and 111) detected with subtype CRF01_AE in the *pol* region and subtype B in the *gag* region.

DISCUSSION

Few data are available regarding the prevalence of viral sexually transmitted infections including HBV and HIV among transgender people in Indonesia. However, several data resources exist regarding the prevalence of HBV and HIV among the general population in several parts of Indonesia (6,9,13,15,25).

Although the prevalence of chronic HBV infection has decreased over the years, an estimated 240 million people have chronic HBV infection worldwide (26). The prevalence of HBV infection varies markedly across different geographical areas of the world. Indonesia is characterized by an intermediate-to-high

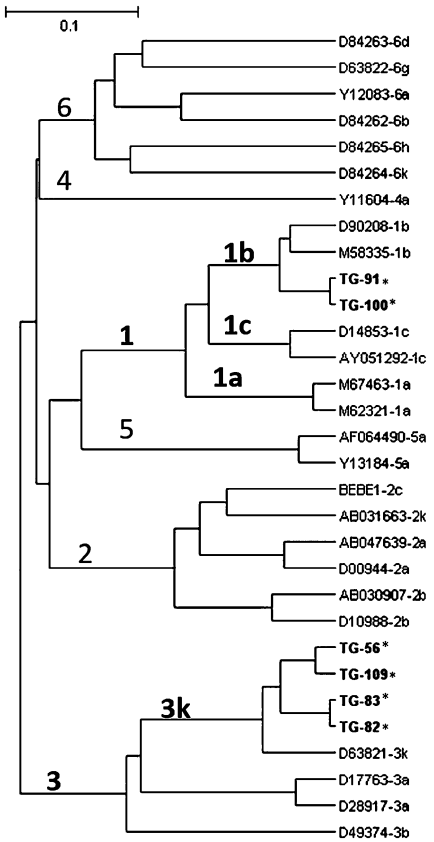

Fig. 3. Unweighted pair group method with arithmetic mean (UPGMA) phylogenetic analysis of HCV isolates from transgender people in Indonesia (shown in bold letter with asterisk) and those from international DNA data bank (indicated with the accession numbers and subtypes), on the basis of partial NS5B sequences. The genotypes and subtypes are indicated on the branches.

HBV rate, and the prevalence of HBsAg has been estimated to be 6–9% in the general population (4). An HBV vaccine has been available since 1982 and has been recommended for use worldwide by the World Health Organization since 1992, but the Indonesian government started the national vaccination program in 1997. The population born before 1997 is not completely vaccinated. Hence, transgender individuals in this population have a higher prevalence of HBV infection as compared to those enrolled in the program. In our study, approximately 5.6% of the samples taken from transgender people in Surabaya, Indonesia, were HBsAg positive, and 9.3% of samples were HBV DNA positive (including 4 samples that were HBsAg negative and anti-HBc positive). The prevalence of HBV infection among transgender individuals is comparable with that of the general population. Four of 19 samples (21.1%; Table 1) showed occult HBV infection; therefore, we strongly recommend that the anti-HBc antibody test be used as a complement to the HBsAg test to routinely screen for HBV.

As shown in Fig. 2, the HBV genotype C isolates formed a separate cluster from subgenotypes C1 to C16. These isolates may belong to subgenotypes other than C1 to C16. Further analysis is needed to verify this possibility. The other isolates belonged to subgenotype B3. This finding is in accordance with those of previous

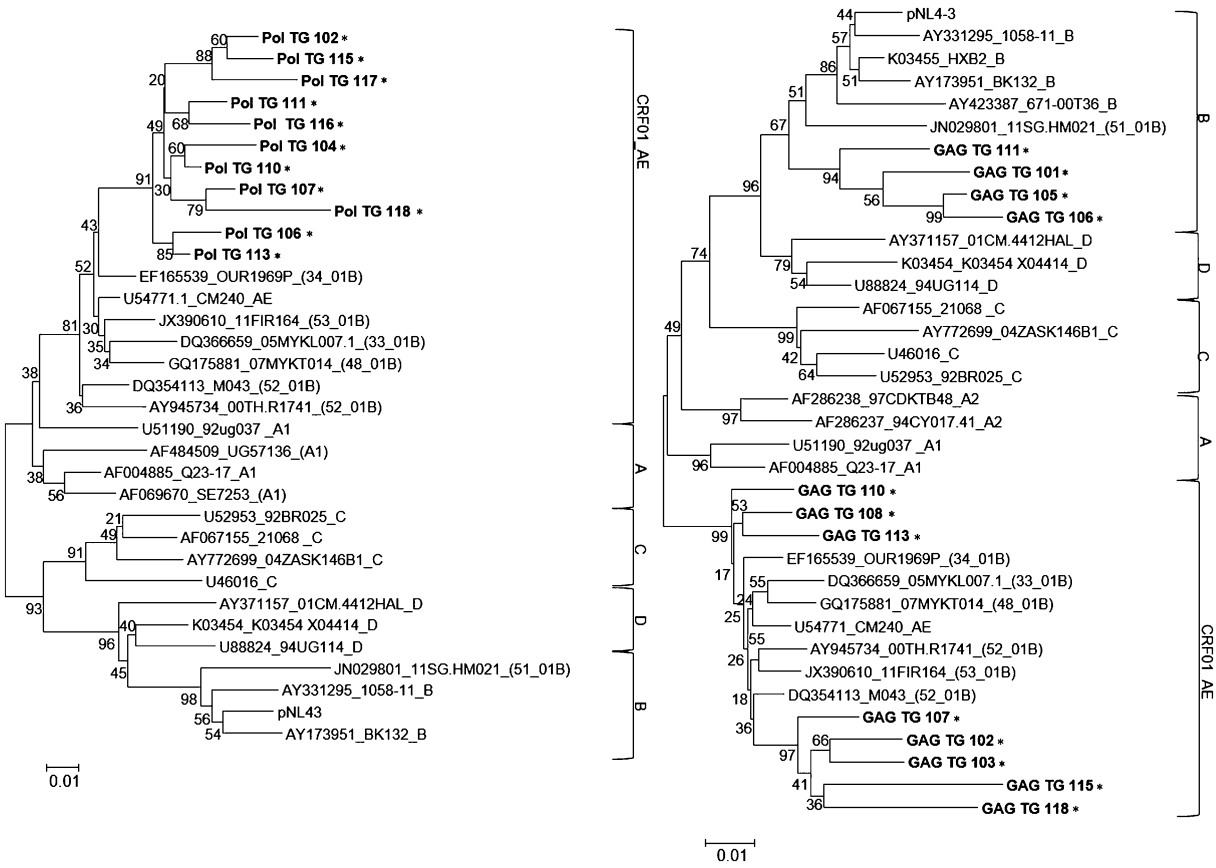

Fig. 4. Phylogenetic analysis of HIV-1 *pol* (A) and *gag* (B) gene sequences from transgender people in Indonesia (code TG; shown in bold letter with asterisks) and reference strains of HIV-1 subtypes. Phylogenetic trees were generated for newly sequenced HIV-1 *pol* and *gag* genes together with the corresponding viral gene 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), and CRF33_01B (33_01B). Bootstrap values are shown when the values are >70.

studies, which showed that most Indonesian HBV isolates, especially among Javanese people, are mostly B3 (27).

Our previous report revealed that >90% of samples obtained from the general population, chronic liver disease patients, and patients on maintenance hemodialysis in Surabaya belong to the genotype B and subtype *adw* (13); this finding is similar to the results found in other parts of Java Island (10,25), and the genotype/subtype C/*adrq*$^+$ was mostly found in the most eastern region of Indonesia (in the Papuan ethnic group) (9,25,28). This study showed at least 30% of the samples (3/9) were of C/*adrq*$^+$, and 2 of them were from Maduranese individuals. However, further study is needed to confirm this finding, since the tested population was very small.

The rates of HCV genotypes/subtypes differ worldwide. We performed sero-epidemiological and molecular epidemiological analyses of HCV among several population groups in Surabaya, Indonesia (18,21,22, 29,30). The rates of anti-HCV antibodies were 2.3%, 76.3%, and 64.7% in healthy blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma, respectively. Another study reported an HCV prevalence of 2.1% in the general Indonesian population (31). Interestingly, a high HCV infection rate of 6.5% was found among transgender people in Surabaya, Indonesia; however, HCV is less likely to be sexually transmitted. Certain mechanisms involved in the sexual transmission of HCV, particularly among partners who engage in behaviors associated with a high risk of virus transmission, might contribute to this high rate of HCV infection among transgender individuals (21,32).

Although the chance of sexually transmission of HCV is <5%, this risk might increase due to certain sexual habits that are often associated with the transgender community, such as multiple sexual partners, anal sex, and traumatic sex (33–36). The transmission risk correlated with anal sex is much higher because it is more likely to cause injury (36).

Interestingly, the subtype distributions of HCV among transgender individuals were 66.6% and 33.3% for HCV-3k and HCV-1b, respectively. These rates might be the highest for HCV-3k reported in Indonesia to date. HCV-2a was previously reported as the most common (52%) among the HCV clones obtained from blood donors in Indonesia, followed by HCV-1b (15%), HCV-1a (7%), and HCV-1c (7%), which is a unique Indonesian subtype (22). In a more recent study of patients with chronic liver disease, Utama et al. reported that HCV 1b (47.3%) was the most prevalent subtype, followed by 1c (18.7%), 3k (10.7%), 2a (10.0%), 1a (6.7%), 2e (5.3%), 2f (0.7%), and 3a

(0.7%) (37). Among people with HCV-HIV co-infection in Surabaya, those who were positive for anti-HCV antibodies showed HCV-1a (31.5%) as the predominant subtype, followed by 3a (23.3%), 1c (10.9%), 1b (9.6%), 3k (4.1%), 2a (1.4%), 4a (1.4%), and genotype 1 (16.4%) (19). Of those who had HCV-HIV co-infection but were negative for anti-HCV antibodies, 26 individuals (38.2%) had HCV RNA, and HCV-3a was the most prevalent subtype (50.0%), followed by 3k (23.1%), 1c (15.4%), and 1b (11.6%). The distribution of various genotypes/subtypes showed geographic variation. Genotype 3 originated from and is mostly found in South Asia (38). In addition, it accounts for >70% of the isolates in India. The distribution of HCV genotypes/subtypes also reflects the route of transmission. We found a relatively high prevalence of genotype 3, including 3k, among people with sexually transmitted HIV-HCV co-infection (18). In our study, the high prevalence of genotype 3 was probably due to sexual activity.

The prevalence of HIV in Indonesia ranges from 0.3% to 0.7%, which is below the global prevalence range of 0.7–0.8% (39,40). However, this low prevalence is because of the uneven distribution of HIV cases in Indonesia. The number of HIV cases in East Java, where Surabaya contributes the largest percentage, is the second highest in Indonesia after Jakarta (the capital city) (41,42). The prevalence of HIV in our study is remarkable because it exceeds the high (12%) prevalence of HIV among female sex workers in Surabaya (6). The genomic fragment of CRF01_AE has been identified as the predominant HIV-1 subtype in South East Asia, including Malaysia, Thailand, and Taiwan (43–45) as well as Jakarta, Surabaya, and Bali in Indonesia (6,46). Our results are consistent with those of previous findings. Remarkably, based on RIP and phylogenetic tree analysis for both *pol* and *gag* genes (Fig. 4), 2 samples were identified as subtype B, and the other 2 were identified as subtype CRF01_AE in the *pol* region, but subtype B in the *gag* region.The diversity of genotype distribution in these samples is predicted to be a new and unique recombinant. This result is similar to that of a previous study of subtype CRF54_01B from Malaysia (47), whereas as reported before, the B subtype is not common in Indonesia. The finding of various subtypes and recombinants in this study indicates the high diversity of the HIV-1 prevalence in Surabaya, Indonesia.

In conclusion, we found a high prevalence (9.3%) of HBV infection among transgender individuals in Surabaya, Indonesia. This rate was as high as that in the adult population in Indonesia. Interestingly, 33.3% of samples were of subtype HBV C/*adrq*$^+$, and 66.7% were Maduranese individuals. C/*adrq*$^+$ was uncommon among the general population in Surabaya. Although HCV is less frequently sexually transmitted, we found that 6.5% of the samples were infected with HCV. Interestingly, HCV-3k was the predominant subtype. Sexual habits that deviate from the norm are associated with a high risk of HCV infection. A very high prevalence of HIV was also found among transgender individuals, and the HIV subtype CRF01_AE was the predominant isolate. Owing to the high rates of HBV, HCV, and HIV infection among transgender people, we strongly recommend routine screenings for these 3 infections.

Acknowledgments The authors are grateful to all of the participants who provided blood samples for this study. We also thank the Community Health Center at Perak District, Surabaya, for their cooperation. A grant from the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) and the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, supported this study.

Conflict of interest None to declare.

REFERENCES

1. Carobene M, Bolcic F, Farias MSDR, et al. HIV, HBV, and HCV molecular epidemiology among trans (transvestites, transsexuals, and transgender) sex workers in Argentina. J Med Virol. 2014;86:64-70.
2. Russi JC, Serra M, Vinoles J, et al. Sexual transmission of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus type 1 infections among male transvestite commercial sex workers in Montevideo, Uruguay. Am J Trop Med Hyg. 2003; 68:716-20.
3. Dienstag JL. Acute viral hepatitis. In: Longo D, Fauci A, Kasper D, et al. editors. Harrison's Principles of Internal Medicine. 18th edition. New York: McGraw Hill; 2012. p. 2537-2557.
4. Thedja MD. Genetic diversity of hepatitis B virus in Indonesia: epidemiological and clinical significance. Utrecht: Utrecht University. 2012;1:3-4.
5. The Joint United Nations Programme on HIV/AIDS. UNAIDS report on the global AIDS epidemic 2012. Geneva, Switzerland. Available at <http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_with_annexes_en.pdf>. Accessed October 1, 2014.
6. Kotaki T, Khairunisa SQ, Sukartiningrum SD, et al. High prevalence of HIV-1 CRF01_AE viruses among female commercial sex workers residing in Surabaya, Indonesia. PloS One. 2013;8: e82645.
7. Darmadi S, Soetjipto, Handajani R, et al. Hepatitis C virus infection-associated markers in sera from blood donors in Surabaya, Indonesia. Microbiol Immunol. 1996;40:401-5.
8. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5:558-67.
9. Lusida MI, Nugrahaputra VE, Soetjipto, et al. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. J Clin Microbiol. 2008;46:2160-6.
10. Rinonce HT, Yano Y, Utsumi T, et al. Hepatitis B and C virus infection among hemodialysis patients in Yogyakarta, Indonesia: prevalence and molecular evidence for nosocomial transmission. J Med Virol. 2013;85:1348-61.
11. Sastrosoewignjo RI, Sandjaja B, Okamoto H. Molecular epidemiology of hepatitis B virus in Indonesia. J Gastroenterol Hepatol. 1991;6:491-8.
12. Kramvis A, Kew M, Francois G. Hepatitis B virus genotypes. Vaccine. 2005;23:2409-23.
13. Lusida MI, Surayah, Sakugawa H, et al. Genotype and subtype analyses of hepatitis B virus (HBV) and possible co-infection of HBV and hepatitis C virus (HCV) or hepatitis D virus (HDV) in blood donors, patients with chronic liver disease and patients on hemodialysis in Surabaya, Indonesia. Microbiol Immunol. 2003; 47:969-75.
14. Gutierrez M, Tajada P, Alvarez A, et al. Prevalence of HIV-1 non-B subtypes, syphilis, HTLV, and hepatitis B and C viruses among immigrant sex workers in Madrid, Spain. J Med Virol. 2004;74:521-7.
15. Juniastuti, Aksono EB, Utsumi T, et al. Analyses of precore and core promoter mutations of hepatitis B virus in patients with chronic hepatitis B in Surabaya, Indonesia. Microbiol Indones. 2010;4:143-8.
16. Akkarathamrongsin S, Praianantathavorn K, Hacharoen N, et al. Geographic distribution of hepatitis C virus genotype 6 in Thailand. J Med Virol. 2010;82:257-62.
17. Utsumi T, Yano Y, Lusida MI, et al. Detection of highly prevalent hepatitis B virus co-infection with HIV in Indonesia.

Hepatol Res. 2013;43:1032-9.
18. Juniastuti, Utsumi T, Nasronudin, et al. High rate of seronegative HCV infection in HIV-positive patients. Biomed Rep. 2014; 2:78-94.
19. Norder H, Courouce AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004;47:289-309.
20. Kleter GEM, van Doorn LJ, Brouwer JT, et al. Sequence analysis of the 5′ untranslated region in isolates of at least four genotypes of hepatitis C virus in the Netherlands. J Clin Microbiol. 1994;32:306-10.
21. Juniastuti, Utsumi T, Aksono EB, et al. Predominance of precore mutations and clinical significance of basal core promoter mutations in chronic hepatitis B virus infection in Indonesia. Biomed Rep. 2013;1:522-8.
22. Soetjipto, Handajani R, Lusida MI, et al. Differential prevalence of hepatitis C virus subtypes in healthy blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma in Surabaya, Indonesia. J Clin Microbiol. 1996;34: 2875-80.
23. Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731-9.
24. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111-20.
25. Utsumi T, Lusida MI, Yano Y, et al. Complete genome sequence and phylogenetic relatedness of hepatitis B virus isolates in Papua, Indonesia. J Clin Microbiol. 2009;47:1842-7.
26. Centers for Disease Control and Prevention. Travel Yellow Book. Chapter 3; Infectious diseases related to travel: hepatitis B. Available at <http://wwwnc.cdc.gov/travel/yellowbook/2014/chapter-3-infectious-diseases-related-to-travel/hepatitis-b>. Accessed October 2, 2014.
27. Heriyanto DS, Yano Y, Utsumi T, et al. Mutations within enhancer II and BCP regions of hepatitis B virus in relation to advanced liver diseases in patients infected with subgenotype B3 in Indonesia. J Med Virol. 2012;84:44-51.
28. Juniastuti, Utsumi T, Nugrahaputra VE, et al. Another novel subgenotype of hepatitis B virus genotype C from papuans of highland origin. J Med Virol. 2011;83:225-34.
29. Hotta H, Handajani R, Lusida MI, et al. Subtype analysis of hepatitis C virus in Indonesia on the basis of NS5b region sequences. J Clin Microbiol. 1994;32:3049-51.
30. Lusida MI, Nagano-Fujii M, Nidom CA, et al. Correlation between mutations in the interferon sensitivity determining region on NS5A protein and viral load of hepatitis C virus subtypes 1b, 1c, and 2a. J Clin Microbiol. 2001;39:3858-64.
31. Sulaiman HA, Julitasari, Sie A, et al. Prevalence of hepatitis B and C viruses in healthy Indonesian blood donors. Trans R Soc Trop Med Hyg. 1995;89:167-70.
32. Mendes Correa MC, Barone AA, Gianini RJ. Risk factors associated with hepatitis C among patients co-infected with human immunodeficiency virus: a case control study. Am J Trop Med Hyg. 2005;72:762-7.
33. Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med. 1999;341:556-62.
34. Abou-Setta AM. Transmission risk of hepatitis C virus via semen during assisted reproduction: how real is it? Hum Reprod. 2004; 19:2711-7.
35. Tahan V, Karaca C, Yildirim B, et al. Sexual transmission of HCV between spouses. Am J Gastroenterol. 2005;100:821-4.
36. Villena EZ. Transmission routes of hepatitis C virus infection. Annals of Hepatology. 2006;5:S12-4.
37. Utama A, Tamia NP, Dhenni R, et al. Genotype diversity of hepatitis C virus (HCV) in HCV-associated liver disease patients in Indonesia. Liver Int. 2010;30:1152-60.
38. Messina JP, Humphreys I, Flaxman A, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015; 61:77-87.
39. The Joint United Nations Programme on HIV/AIDS. Epidemiological fact sheet on HIV and AIDS. Available at <http://www.unaids.org/sites/default/files/epidocuments/IDN.pdf>. Accessed January 31, 2015.
40. World Health Organization. Prevalence of HIV among adults aged 15–49 years, 2013. Available at <http://www.who.int/gho/hiv/hiv_013.jpg?ua=1>. Accessed February 1, 2015.
41. Ministry of Health East Java Province Indonesia. Profil Kesehatan Provinsi Jawa Timur Tahun 2012. Available at <http://www.depkes.go.id/resources/download/profil/PROFIL_KES_PROVINSI_2012/15_Profil_Kes.Prov.JawaTimur_2012.pdf>. Accessed January 23, 2015.
42. Ministry of Health, Indonesia. Cases of HIV/AIDS in Indonesia reported through September 2014. Available at <http://www.spiritia.or.id/Stats/StatCurr.pdf>. Accessed January 23, 2015.
43. Auwanit W, Isarangkura-Na-Ayuthaya P, Kasornpikul D, et al. Detection of drug resistance-associated and background mutations in human immunodeficiency virus type 1 CRF01_AE protease and reverse transcriptase derived from drug treatment-naive patients residing in central Thailand. AIDS Res Hum Retroviruses. 2009;25:625-31.
44. Mohamad S, Derisa ZZ, Yusoffb NK, et al. Assessing subtypes and drug resistance mutations among HIV-1 infected children who failed antiretroviral therapy in Kelantan, Malaysia. Braz J Infect Dis. 2012;16:284-8.
45. Jack BC, Lai YO, Takebe Y, et al. Molecular detection of HIV-1 subtype B, CRF01_AE, CRF33_01B, and newly emerging recombinant lineages in Malaysia. Am J Trop Med Hyg. 2015;92: 507-12.
46. Sahbandar IN, Takahashi K, Djoerban Z, et al. Current HIV type 1 molecular epidemiology profile and identification of unique recombinant forms in Jakarta, Indonesia. AIDS Res Hum Retroviruses. 2009;25:637-46.
47. Ng KT, Ong LY, Takebe Y, et al. Genome sequence of a novel HIV-1 circulating recombinant form 54_01B from Malaysia. J Virol. 2012;86:11405-6.