Torque Teno Virus in HIV-infected transgender in Surakarta, Indonesia

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Abstract. Torque Teno Virus (TTV) is a circular single-stranded DNA virus that may co-infected with human immunodeficiency virus (HIV), especially in the high-risk community e.g. the transgender performing high-risk behavior. TTV shows an increased viremia in HIV patients and maybe influence the HIV clinical progression. Blood samples collected from transgender performing high-risk behavior in Surakarta were tested by serological and molecular assays to detect the presence of HIV infection. The blood samples with HIV positive status were then tested by a nested polymerase chain reaction (PCR) to detect the presentation of TTV DNA. The amplified PCR products were molecularly cloned and subjected to sequence analysis. TTV DNA was detected in 40.0% HIV-positive samples. The molecular characterization revealed that the most prevalent was genogroup 3, followed by genogroup 2 and 1, respectively. TTV was detected in HIV-infected transgender performing high-risk behavior in Surakarta with high infection rate.

1. Introduction
Torque Teno Virus (TTV), is a circular single-stranded DNA virus member of Anelloviridae family considered a worldwide distributed emergent virus. The pathogenicity of TTV remains unclear, however, it has been constantly associated with concurrent infections [1-4]. TTV has been detected in almost every human tissue type or body fluid reaching near 100% prevalence [5] and frequently found co-infected with human immunodeficiency virus (HIV) [5-13]. TTV showing an increased viremia in HIV patients and TTV load may be used as a surrogate marker of immune function related to B cells exhaustion and impaired viral immune surveillance in HIV patients. TTV might play a role in the development of liver disease in HIV patients, such as the patients coinfected with HIV and HCV, and maybe influence the HIV clinical progression [10-11].

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The transgender community is at a higher risk for substance use, sexually transmitted diseases (STDs), bullying, isolation, rejection, anxiety, and depression as compared to the general population. Transgender usually receive poor quality of care due to stigma, lack of healthcare providers' awareness, and insensitivity to the unique needs of this community [12]. Previously, our research group (A-IGIC/A-Infection, Genomics, Immunology, & Cancer research group) reported the circulation of TTV in Indonesia [4,6-8,14]. The circulation of HIV in transgender living in Surakarta also already reported by our research group [6-8,15-18]. The present study describes the circulation of TTV in HIV-infected transgender performing high-risk behavior in Surakarta.

2. Materials and Methods
Since 2009, our group has been conducting a molecular epidemiology study of human blood-borne pathogens in Central Java, Indonesia [4, 6-8,14-24,26-31]. Epidemiological-clinical data and blood samples were collected from the high-risk communities, including that of the transgender performing high-risk behavior in Surakarta. 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 procedures were conducted according to the principles of the Declaration of Helsinki.

The study participants completed an interviewer-administered questionnaire by a specially trained interviewer that assessed general characteristics associated with blood-borne virus infections, as reported previously [6,14,17,24]. All blood samples were subjected to human blood-borne pathogens detection by immunological and molecular assays, as reported previously [4,6-8,14-31]. All blood samples were screened by immunological and molecular assays for HIV-infection detection. 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 (Bio M’erieux, Marcy l’Etoile, France). Viral nucleic acid was extracted from plasma by using High Pure Viral Nucleic Acid Kit (Roche Life Science, Manheim, Germany) according to the manufacturer’s instructions. 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). To detect the presence of HIV RNA, a portion of the HIV gag gene encoding the p24 region was amplified by nested PCR using the cDNA as template, by the Fast Start High Fidelity PCR System (Roche Life Science). From transgender performing high-risk behavior in Surakarta in 2009-2011, a total of 40 blood samples were found positive for HIV and then used for the present study.

The molecular detection techniques for TTV were performed as described previously [14]. TTV DNA was extracted from whole blood by using High Pure PCR Template Preparation Kit (Roche Life Science). TTV DNA was amplified by a nested PCR amplification using the Fast Start High Fidelity PCR System (Roche Life Science). 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. To prevent PCR contamination, the reagent preparation, sample processing, and nested PCR assays were performed in rooms separate from where the amplified products were analyzed. Aerosol-resistant pipette tips were used throughout the assays. The PCR products were subjected to electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination. The amplicons were purified, subcloned, and sequenced. All samples were tested at least twice.

3. Results and Discussion
During 2009-2011, 40 transgender performing high-risk behavior in Surakarta were found positive for HIV infection. All participants identified themselves as not married. The majority of the participants (33/40, 82.5%) were informal employees, and 7 participants were work as commercial sex workers. All participants lived in Surakarta and nearby cities (Table 1). None of the participants knew anything about TTV but all participants knew about the prevention and transmission routes of HIV.
Approximately 30.0% (12/40) of participants had had an HIV test, and 25.0% (10/40) had undergone STI screening; however, none of them had been screened for liver function and hepatitis. None of the participants had ever had intercourse with a person whom they had known to have a history of infection with HIV or hepatitis. Unfortunately, all participants never asked about their sex partner’s HIV or STI or hepatitis infection status. Regarding other risk behaviors, 10.0% (4/40) of the participants had been an inmate, 70.0% (28/40) had abused non-injected drugs, and 25.0% (10/40) had abused injected drugs. Overall, 15 participants (37.5%) had a tattoo, 40 (100.0%) had been pierced, and 10 (25.0%) had both a tattoo and a piercing. All participants had shared needle history for tattoo and or piercing. All participants also had sexual activities (oral and anal) without condom history. There were no reports about contact with blood, being pricked with a needle contaminated with blood, blood transfusions, dialysis, history of traumatic injury, or transplantation. Almost all participants had been a circumcised (35/40, 87.5%) and had a smoking history (33/40, 82.5%). Alcohol intoxication was self-reported by 25 participants (62.5%). The health status was good for all of the participants in the time of data collection.

| Table 1. Socio Demographic Data of HIV-Infected Transgender Performing High-Risk Behavior in Surakarta, Indonesia |
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| Factors | Total N (40) | % (100) |
| Age (years) | | |
| 19-29 | 24 | 60.0 |
| 30-39 | 16 | 40.0 |
| 40-50 | 0 | 0.0 |
| Marital status | | |
| Married | 0 | 0.0 |
| Ever married | 0 | 0.0 |
| Not married | 40 | 100.0 |
| Education | | |
| Undergraduate | 0 | 0.0 |
| Senior high school | 15 | 37.5 |
| Junior high school | 15 | 37.5 |
| Elementary school | 10 | 25.0 |
| Occupation as sex worker | 7 | 17.5 |
| Occupation as non-sex worker | | |
| Unemployed | 20 | 50.0 |
| Employee | 13 | 32.5 |
| Monthly Income | | |
| <100 USD | 30 | 75.0 |
| >100 USD | 10 | 25.0 |
| Ethnicity | | |
| Javanese | 32 | 80.0 |
| Non-Javanese (Ethnic minority) | 8 | 20.0 |
| Residence | | |
| Surakarta | 26 | 65.0 |
| Nearby cities | 14 | 35.0 |

Note: N, the number of participants; USD, US Dollar.

TTV DNA was detected in 40.0% (16/40) samples. The molecular characterization revealed that the most prevalent was genogroup 3 (50.0%, 20/40), followed by genogroup 2 (30.0%, 12/40), and 1 (20.0%, 8/40), respectively, in line with a previous report [14]. Clinical progression of HIV patients leads increase TTV viral load [32], and the TTV viral load itself may associate with patient’s clinical
output [10-11]. The susceptibility of TTV co-infection and its pathogenesis role in Indonesian HIV patients may associate with patient’s polymorphisms status and other co-infection status or the molecular status of the pathogen itself [4,6-8,14-24,26-31].

HIV disproportionately impacts populations that have traditionally suffered from health disparities as in transgender community[33]. Transgender individuals also often experience significant health disparities [34]. In the present study, all transgender infected with HIV were found had high-risk activities (sharing needle for tattoo and or piercing, saliva transfer, and sexual activities without a condom). Co-infection of TTV was also found, indicated the high-risk activities would increase the risk of other pathogens co-infection, therefore, may influence the patient’s clinical output.

4. Conclusions
TTV co-infection was found with high infection rate in HIV-infected transgender performing high-risk behavior in Surakarta. HIV patient with high-risk activities history should be screened for other blood-borne pathogens.

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