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No Increase of Drug-Resistant HIV Type 1 Prevalence Among Drug-Naive Individuals in Northern Vietnam

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Abstract

We reported previously that the prevalence of drug-resistant HIV-1 among antiretroviral therapy (ART)-naive individuals in Northern Vietnam was 2.9% in 2007 and 6.2% in 2008. To investigate the continuing trend of prevalence, we collected plasma samples from 958 individuals in Hai Phong and Hanoi in 2009, extracted viral RNA from HIV-1 antibody-positive samples, and analyzed them genetically. HIV-1 antibody prevalence was 26.8% in injecting drug users ($n=302$), 13.4% in female sex workers ($n=284$), 0.5% in blood donors ($n=206$), and 0.6% in pregnant women ($n=166$). All HIV-1 strains were CRF01_AE. Nonnucleoside reverse-transcriptase inhibitor resistance mutations were found in two (2.0%) of the 102 successfully analyzed cases (one case with the Y181C and one with the K101E). No nucleoside reverse-transcriptase inhibitor resistance or protease inhibitor resistance mutations were detected. The prevalence of circulating ART-resistant HIV-1 in Northern Vietnam did not increase from 2007 to 2009, although the rate of ART coverage did increase.

In Vietnam, the first human immunodeficiency virus type 1 (HIV-1)-infected case was reported in Ho Chi Minh City in 1990,[1] and HIV-1 infection had spread throughout the entire country by 1999.[2] In 2010, an estimated 254,000 people in Vietnam were living with HIV.[3] The most prevalent HIV-1 subtype in Vietnam is CRF01_AE, although subtype B, subtype C, and intersubtype recombinants have also been identified.[4–9] Antiretroviral therapy (ART) has been introduced to Vietnam intensively since 2003. The ART coverage rate among adults increased from 30% in 2007 to 53.7% in 2009,[3] which might induce the appearance and circulation of ART-resistant HIV-1 strains in Vietnam. Previously we conducted studies on the prevalence of drug-resistant HIV-1 strains among ART-naive HIV-1-infected patients in Northern Vietnam, and observed prevalences of 2.9% in Haiphong in 2007[4] and 6.2% in three provinces, including Hanoi, in 2008.[5] In the current study, we investigated the trend in the prevalence of HIV-1 and drug-resistant HIV-1 in Northern Vietnam between 2007 and 2009.

Plasma samples were collected from 958 individuals residing in two cities in Northern Vietnam ($n=738$ from Hai Phong and $n=220$ from Hanoi) in late 2008 to 2009. The study participants were divided into four groups according to their HIV-1-infection risks: injecting drug users ($n=302$), female sex workers ($n=284$: 64 from Hai Phong and 220 from Hanoi), blood donors ($n=206$), and pregnant women ($n=166$). The characteristics of the participants are summarized in Table 1. None had any previous history of ART. Plasma samples were screened for anti-HIV antibody (Determine HIV-1/2; Inverness Medical Japan Co., Ltd., Tokyo, Japan).

HIV-1 RNA was extracted from 100 μl of anti-HIV-1-positive plasma samples using a SMITEST EX-R&D Nucleic Acid Extraction Kit (Genome Science Laboratories, Fukushima, Japan) according to the manufacturer's instructions. The protease- and reverse transcriptase-encoding regions of the HIV-1 *pol* gene (*pol*-PR and *pol*-RT, respectively) were amplified by one-step reverse transcription polymerase chain reaction (RT-PCR; SuperScript III One-step RT-PCR System with Platinum Taq High Fidelity; Invitrogen, Carlsbad, CA), and then by nested PCR using KOD FX (Tokyo, Osaka, Japan) as described previously.[4,5] The amplified PCR products were sequenced directly and/or after cloning, if necessary, and analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sample nucleotide sequences were aligned with HIV-1 subtype reference sequences retrieved from the Los Alamos database, and with previously reported sequences of HIV-1 strains isolated from Southeast Asia and Southern China using Clustal W (version 1.83) with minor manual adjustments. Phylogenetic trees were constructed and visualized as described previously.[4,5] The *pol*-PR (297 base pairs) and *pol*-RT (660 base pairs) nucleotide sequences were translated into the corresponding 99 and 220 amino acids,

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Table 1. Characteristics of the Study Participants and Prevalence of HIV-1 Antibody

Groups	n	*Male/female*	*Mean age (SD)*	*HIV-1 antibody positive (%)* *Current study*	*HIV-1 antibody positive (%)* *Study in 2007*[a]
IDU	302	302/0	32.5 (8.5)	26.8%*	35.9% ($n=760$)*
FSW	284	0/284	28.0 (8.2)	13.4%**	23.1% ($n=91$)**
Blood donors	206	182/24	29.1 (8.3)	0.5%	2.9% ($n=210$)
Pregnant women	166	0/166	26.1 (5.4)	0.6%	0.5% ($n=200$)
Total	958	484/474	29.0 (8.2)	$n=121$	

[a] Ishizaki *et al.*[4]
*$p=0.006$; **$p=0.041$.
IDU, injecting drug users; FSW, female sex workers.

respectively. Using the Stanford University HIVdb sequence analysis program and the International AIDS Society-USA November 2011 list,[10] we analyzed the amino acid sequences for drug resistance-associated mutations.

Of the 958 individuals, 121 individuals were positive for HIV-1 antibody: the prevalence was 26.8% (81/302) in injecting drug users, 13.4% (38/284) in female sex workers, 0.5% (1/206) in blood donors, and 0.6% (1/166) in pregnant women (Table 1). Notably, the HIV-1 prevalence among high-risk groups in Northern Vietnam in 2009 was significantly lower than reported in our previous study in 2007[4]: 35.9% ($n=760$) in injecting drug users ($p=0.006$) and 23.1% ($n=91$) in female sex workers ($p=0.041$). These findings indicate that the HIV-1 epidemic in Northern Vietnam is still in a concentrated stage among high-risk groups such as injecting drug users and female sex workers, with a gradual decrease of HIV-1 prevalence, as observed in other areas of Vietnam.[3] These findings also suggest that the Preventing HIV Project in Vietnam, which targets injecting drug users and female sex workers, may have taken effect to reduce the number of new infections in the main risk groups in Hai Phong.[11,12]

Of the 121 HIV-1 antibody-positive samples, 107 samples were successfully analyzed genetically for *pol*-PR and 102 were successfully analyzed for *pol*-RT. Phylogenetic analysis revealed that all of the isolated strains belonged to HIV-1 CRF01_AE, and most of them formed a large cluster with previously reported sequences from Northern Vietnam and Southern China, as reported previously[4,6] (data not shown). Nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance mutations were observed in two (2.0%) of the 102 successfully analyzed cases: one case with the Y181C mutation and one with the K101E mutation (Table 2). The Y181C and K101E mutations are common drug-resistant mutations observed in CRF01_AE-infected individuals who are failing nevirapine-based therapy.[13] The K101E mutation was first identified among ART-naive individuals in Vietnam. The potential NNRTI resistance mutations, V106I and V179D, were observed in three cases each (2.9%).

No nucleoside reverse-transcriptase inhibitor resistance or major protease inhibitor (PI) resistance mutations were detected; however, the minor PI resistance mutations L10I, L10V, K20I, and A71V were detected in 11 (10.3%), four (3.7%), four (3.7%), and one (0.9%) cases, respectively, as observed previously.[4] The overall prevalences of reverse-transcriptase inhibitor resistance and PI resistance mutations were 2.0% and 0%, respectively. Therefore, the prevalence of circulating ART-resistant HIV-1 in Northern Vietnam slightly decreased from 2.9% in 2007[4] and 6.2% in 2008[5] to 2.0% in 2009 (current study), although the ART coverage rate in Vietnam increased from 30% in 2007 to 53.7% in 2009.[3] Interestingly, the two cases harboring drug-resistant HIV-1 were not injecting drug users from Hai Phong, but female sex workers from Hanoi, while in our previous study in Hai Phong[4] all individuals with drug-resistant HIV-1 were injecting drug users.

This finding might be due to the successful outcome of the HIV prevention programs in Hai Phong, such as harm reduction interventions for injecting drug users, which include distributing clean needles and condoms, as well as methadone maintenance therapy.[3,11] It also suggests that the prevalence of circulating ART-resistant HIV-1 can differ among groups and areas, even within Northern Vietnam. These findings indicate that there is still a need to monitor drug resistance mutations in Northern Vietnam.

GenBank Accession Numbers

The GenBank accession numbers of the sequences reported in this study are AB690876–AB690982 for *pol*-PR and AB690983–AB691084 for *pol*-RT.

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Table 2. Characteristics of Vietnamese Individuals Harboring Antiretroviral Drug Resistance Mutations

Sample ID	*Age/sex*	*City*	*HIV-1 subtype*	*PI resistance mutations*	*RTI resistance mutations* *NRTI*	*RTI resistance mutations* *NNRTI*
HPV2_124_09	30/F	Hanoi	CRF01_AE	none	none	K101E
HPV2_209_09	22/F	Hanoi	CRF01_AE	none	none	Y181C

PI, protease inhibitor; RTI, reverse transcriptase inhibitor; NRTI, nucleoside RTI; NNRTI, nonnucleoside RTI.

Author Disclosure Statement

No competing financial interests exist.

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