Short Communication

Emerging Transmitted HIV Type 1 Drug Resistance Mutations Among Patients Prior to Start of First-Line Antiretroviral Therapy in Middle and Low Prevalence Sites in China

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Abstract

It is known that transmitted drug resistance (TDR) will most likely emerge in regions where antiretroviral therapy (ART) has been widely available for years. However, after a decade of rapid scale-up of ART in China, there are few data regarding TDR among HIV-infected patients prior to initiating first-line ART in China. A prospective, observational cohort study was performed at sentinel sites in five provinces or municipalities. Study participants were recruited at the county- or city-level centers for disease control (CDCs), during routine monitoring visits following referral from diagnosing parties (e.g., hospitals). Each province or municipality recruited 140 patients through sequential sampling throughout the 2011 calendar year. A total of 627 eligible subjects were included in the analysis. The median CD4⁺ cell count was 206 cells/ml at the baseline survey. The majority of patients (93.5%) had plasma HIV viral load $\geq 1,000$ copies/ml. Of the 627 patients, 17 (2.7%) had drug resistance mutations for any type of HIV drugs. The prevalence of drug resistance mutations to non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs (8/627, 1.3%) was higher than to nucleoside reverse transcriptase inhibitor (NRTI) drugs (5/627, 0.8%) and protease inhibitor (PI) drugs (4/627, 0.6%). A logistic regression model showed that the only predictive factor was the route of infection through homosexual intercourse, i.e., men who have sex with men (MSM) status. As HIV prevalence is rising rapidly among Chinese MSM, it is essential to continue surveying this risk group and related high-risk populations with low awareness of HIV, and to develop new public health interventions that help to reduce the spread of drug-resistant HIV.

Introduction

TRANSMITTED DRUG RESISTANCE (TDR) mutations in the HIV-1 virus represent a challenge for patient treatment, as mutations can reduce the efficacy of antiretroviral therapy (ART) and may impact clinical outcomes. The emergence of TDR in resource-limited settings is a considerable threat to the global scale-up of ART. It is known that TDR will most likely emerge in regions where ART has been widely available for years. However, after a decade of rapid scale-up of ART in China, there are few data regarding TDR among HIV-infected patients prior to initiating first-line ART treatment in China.

This study examined the rates of TDR in 2011 at multiple sentinel locations across China representing diverse patient demographics.

Materials and Methods

Study population

We establish a prospective, observational cohort for HIV drug resistance surveillance at 11 sentinel sites in five provinces or municipalities: Sichuan Province (Butuo county), Hunan Province (Hengyang City), Chongqing Municipality

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(Shapingba, Jiulongpo, and Yuzhong districts), Jiangsu Province (Nanjing, Suzhou, and Nantong cities), and Guizhou Province (Guiyang, Tongren, and Dujun cities). Study participants were recruited at the county- or city-level Center for Disease Control (CDC) during routine monitoring visits following referral from HIV testing clinics/hospitals. Criteria for enrolling in the study were naive for ART, aged 18 years or older, agreeing to initiate ART in the National Free Antiretroviral Treatment Program (NFATP), and willing to provide informed consent. Each province or Municipality recruited 140 patients through sequential sampling in 2011. This study reported the baseline data of this cohort.

Laboratory tests

Venous blood samples were collected for testing CD4⁺ T-lymphocyte count (CD4 count), HIV viral load, and HIV drug resistance mutations. CD4 count was tested using flow cytometry (FACSCalibur, BD Company) within 24 h after specimen collection in local CDCs. Plasma was isolated and stored frozen at -80°C in local CDCs before transferring to the National Center for AIDS/STD Control and Prevention (NCAIDS) in Beijing City for testing viral load and drug resistance mutations. Plasma HIV RNA was quantified with real-time NASBA (NucliSense Easy Q, bioMérieux, France) or with COBAS (Roche Applied Biosystems, Germany) according to the manufacturers' recommendations. HIV drug resistance genotyping was performed at NCAIDS using an in-house polymerase chain reaction (PCR) protocol. Drug resistance mutation analysis and viral subtype determination were performed on a 1.3-kb section of the HIV *pol* gene using the Stanford University HIV Drug Resistance Database online sequence analysis tool (http://hivdb.stanford.edu/pages/algs/sierra_sequence.html). We included mutation results that conferred low-, intermediate-, and high-level resistance.^{1,2}

Statistical analysis

The rates of drug resistance mutations to individual drug and to any categories of ART drugs were calculated. A logistic regression model was used to explore factors associated with TDR. Statistical significance was defined as a p -value < 0.05 . All statistical analyses were performed using SAS 9.1 software (SAS Institute, Cary, NC).

Results

Characteristics of participants

Of the 729 study participants recruited, 11 could not be found in the NFATP patient database and were therefore excluded. Additional 91 participants were excluded from the analysis due to the failure of HIV RNA sequencing. Among the remaining 627 eligible pre-ART subjects, 484 (77.2%) were male. The median age was 36 years (range 18–78). Marital status was single (24.1%), married or cohabiting (61.6%), and other (14.4%). Education distribution was no schooling (18.5%), primary school (12.4%), middle school (26.3%), high school (20.7%), and college or above (22.0%). The risks of HIV infection were intravenous drug use (24.7%), homosexual contacts (26.0%), and heterosexual contacts (43.4%). Participants from five provinces had different risks of HIV infection: injection drug use accounting for over 70% in Sichuan Pro-

vince (injection drug use, 73.5%), Hunan (heterosexual, 59.5%), Guizhou (heterosexual, 50.0%), Jiangsu (homosexual, 50.0%; heterosexual, 46.4%), and Chongqing (homosexual, 49.2%; heterosexual, 35.6%).

The median CD4 count was 206 cells/ml, and the proportion of patients with CD4 count of 0–99, 100–199, 200–349, 350–499, and ≥ 500 cells/ml was 24.7%, 22.3%, 38.8%, 11.5%, and 2.7%, respectively. The majority of patients (586/627, 93.5%) had plasma HIV viral load $\geq 1,000$ copies/ml. Finally, 291 patients (46.4%) were infected with subtype CRF07_{BC}, while 271 were subtype CRF01_{AE} (43.2%); the remaining (10.4%) were subtypes B, C, or CRF08_{BC}.

HIV drug resistance mutations

Of the 627 patients, 17 (2.7%) had drug resistance mutations for any type of HIV drugs. All 17 subjects with drug resistance mutations had a median HIV viral load of 84,600 (range, 1,400–1,300,000) copies/ml. The prevalence of drug resistance mutations to nonnucleoside reverse transcriptase inhibitor (NNRTI) drugs (8/627, 1.3%) was higher than that to nucleoside reverse transcriptase inhibitor (NRTI) drugs (5/627, 0.8%) and protease inhibitor (PI) drugs (4/627, 0.6%). The detailed rates for individual drug are shown in Table 1.

TABLE 1. DISTRIBUTION OF MUTATION ALLELES AMONG 17 PATIENTS WITH HIV-1 DRUG RESISTANCE

<i>Antiretroviral drugs</i>	<i>Mutation alleles (%)</i>	<i>Number of mutations [n (%)]</i>
Overall		17 (100.0)
Any nonnucleoside reverse transcriptase inhibitors (NNRTIs)	K101E (5.9) E138EK (5.9) K103N (5.9)	8 (47.1)
Efavirenz (EFV) ^a	Y188L (5.9)	6 (35.3)
Nevirapine (NVP) ^a	L100*IKL (5.9)	8 (47.1)
Delavirdine (DLV)	R138K (5.9)	8 (47.1)
Etrivine (ETV)	V106A/M (11.8)	3 (17.7)
Any nucleoside reverse transcriptase inhibitors (NRTIs)	K65KR (11.8) M184V (17.7)	5 (29.4)
Lamivudine (3TC) ^a		5 (29.4)
Azidothymidine (AZT) ^a		0 (0.0)
Stavudine (D4T) ^a		2 (11.8)
Didanosine (DDI) ^a		2 (11.8)
Abacavir (ABC)		2 (11.8)
Emtricitabine (FTC)		5 (29.4)
Tenofovir (TDF) ^a		2 (11.8)
Any protease inhibitors (PIs)	I54IT (5.9) I84IV (5.9)	4 (23.5)
Atazanavir (ATV)		1 (5.9)
Darunavir (DRV)	M46LM (5.9)	0 (0.0)
Fosamprenavir (FPV)	N88DN (5.9)	1 (5.9)
Indinavir (IDV)		1 (5.9)
Lopinavir + ritonavir (LPV) ^a		1 (5.9)
Nelfinavir (NFV)		4 (23.5)
Saquinavir (SQV)		2 (11.8)
Tipranavir (TPV)		1 (5.9)

^aThese drugs have been provided through the National Free Antiretroviral Treatment Program (NFATP), and the others were not.

Factors associated with HIV TDR

TDR was defined as resistance mutations to the drugs that have been included in the NFATP regimens as of the date of participant enrollment. For mutations against drugs that were not included in the NFATP and were not widely used in China, we assumed they were due to HIV polymorphism rather than TDR. In a logistic regression model, only one demographic factor was associated with TDR: risk of infection [OR=2.9, 95% confidence interval (CI): 1.0–8.5, for homosexual contacts (MSM) versus other risks]. The prevalence rates of TDR among patients infected through homosexual contacts and other risks were 4.3% (7/163) and 1.5% (7/464), respectively. Other demographic and disease factors have no statistically significant association with TDR, including sex, age, marital status, education, occupation, location of residence, ART history of spouse or regular sexual partner, disease stage, HIV subtype, and CD4 count.

Discussion

The overall rate of TDR from 11 sentinel sites across five Chinese provinces and municipalities in 2011 was 2.7%. It is lower than 3.8% from our previous national survey in 2004–2005.³ One possible reason for this difference is that the current survey was conducted in the areas where ART coverage rates were low, and treatment duration was short, because the NFATP was started in 2002 in provinces with plasma donation-associated HIV infection, and was scaled-up in other provinces after 2004.^{4,5} The cumulative reported number of HIV/AIDS cases for Sichuan, Hunan, Chongqing, Jiangsu, and Guizhou at the end of 2011 were 48,357, 13,187, 12,150, 5,551, and 12,414, respectively, and the cumulative number of treated patients for the five provinces/municipalities were 10,741 (22.2%), 4,490 (34.0%), 2,991 (24.6%), 2,402 (43.3%), and 2,457 (19.8%), respectively. The NFATP was scaled-up widely in Hunan after 2006 and in the other four provinces/municipalities after 2008 [NFATP database].

In investigating predictive factors for TDR transmission, we found that the only predictor was risk of infection by homosexual intercourse. This is likely due to a large sexual networks among homosexual men (MSM) in China.^{6–8} Meanwhile, Chinese MSM often get married due to social pressure and therefore they may have wives or female sexual partners. Therefore, those with TDR will pose a risk of transmitting these HIV subtypes to both their male and female sexual partners.⁹

In summary, as the availability of antiretroviral drugs continues to expand in China, monitoring of TDR among the treatment-naïve population should be conducted paralleling the monitoring of those on ART. As TDR prevalence was higher among MSM, and MSM has become a major population at HIV risk, MSM should become one of the priority populations for TDR surveillance.

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Author Disclosure Statement

No competing financial interests exist.

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