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Original Article

HIV drug resistance and related factors in patients receiving second-line combination antiretroviral therapy in rural China

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Abstract: Objective: This study aims to understand the rates of and analyze the risk factors for HIV drug resistance (HIVDR) among patients on second-line combination antiretroviral therapy (cART). Methods: Patients receiving cART between January 2007 and December 2009 whose regimens were changed to second-line cART for at least 6 months, were included in this cross-sectional study, conducted from 2013 to 2014. Patients completed a questionnaire, and blood samples were collected to measure CD4 cell count and HIV viral load (VL), and to perform HIV resistance genotyping. Factors associated with HIVDR were identified using multivariate logistic regression. Results: Of the 122 patients included in this study, 33 (27%) had virologic failure (VL >1000 copies/mL). In these 33 patients, HIVDR was found to non-nucleoside reverse transcriptase inhibitors (42.4%), nucleoside reverse transcriptase inhibitors (36.4%), and to both of these drug classes (24.2%). Virologic failure was associated with a shorter duration of first-line cART and lower CD4 cell counts, whereas HIVDR was associated with higher CD4 cell counts (OR 10.62; 95% CI 2.46, 45.91). Conclusions: The prevalence of HIVDR was high in the study population, representing a challenge to the scaling up of treatment in the country. Adherence to cART should be given with greater attention.

Keywords: HIV, second-line antiretroviral therapy, drug resistance, cross-sectional study

Introduction

By the end of 2014, an estimated 36.9 million people were living with human immunodeficiency virus (HIV) infection globally, with 15.8 million on combination antiretroviral therapy (cART) [1]. The rapid increase in the use of cART over the past decade has dramatically decreased morbidity and mortality due to acquired immunodeficiency syndrome (AIDS), and has decreased the number of new HIV infections [2]. However, the eventual development of HIV drug resistance (HIVDR) is one of the strongest predictors of treatment failure [3]. A number of patients have developed HIVDR to first-line cART regimens, and a growing number of patients in developing countries are on second-line therapy [4-6].

The National Free Antiretroviral Treatment Program (NFATP) has been available in China since 2003. In this program, first-line regimens include two nucleoside reverse transcriptase inhibitors (NRTIs)-zidovudine (AZT) or stavudine (D4T) plus didanosine (DDI) or lamivudine (3TC)-plus one non-nucleoside reverse transcriptase inhibitor (NNRTI)-nevirapine (NVP) or efavirenz (EFV). HIVDR inevitably occurs with the use of first-line regimens, affecting an increasing number of patients. According to a meta-analysis of longitudinal cohort studies conducted in China, the prevalence of HIVDR was 10.8% after 12 months of cART and 80.6% after 72 months of first-line cART [7].

In 2009, China introduced the following second-line cART regimen for patients who failed

first-line therapy: tenofovir (TDF)+3TC+ritonavir-boosted lopinavir (LPV/r) [8]. Virologic failure rates after 6 months of second-line cART of 21.8% and 17.3% have been reported in resource-limited settings [9] and in China [10], respectively. For HIV-infected Chinese patients failing second-line therapy, treatment options are largely nonexistent. Thus, there is a need to understand the rates of and reasons for HIVDR in those taking second-line cART. This cross-sectional study aimed to investigate HIVDR in patients taking second-line cART and to determine the rate of HIVDR and factors related to its development, in order to limit its occurrence and to forecast the need for treatment options beyond second-line therapy.

Materials and methods

Study design and population

This cross-sectional study was conducted in the Henan province in China, which has a high prevalence of HIV infection, between December 2013 and July 2014. HIV-infected patients aged >18 years who had received first-line cART between January 2007 and December 2009 and who had switched to and had been taking second-line cART for at least 6 months, were included. Patient information and blood samples were collected; the latter were used to measure CD4 cell count and HIV viral load (VL) level, and to perform HIV drug-resistance genotyping.

Data collection and variables

Patient information was obtained by the investigator using a questionnaire either during a face-to-face interview or from medical records. Peripheral venous blood was obtained from each patient and stored in an EDTA-3 K tube containing anticoagulants. CD4+T lymphocyte count was measured within 24 hours. Blood for measurement of VL and for drug resistance testing was centrifuged for 15 minutes; the plasma was stored at -80°C until the tests were performed.

Laboratory analysis

If the sample VL was >1000 copies/mL, HIV-1 viral RNA was extracted from a 140 uL plasma sample. Reverse transcription-polymerase chain reaction (RT-PCR) was performed with a one-step RNA PCR Kit (AMV) within 4 h: 1300 base pairs of HIV-1 reverse transcriptase and protease genes were amplified with nested PCR, including the whole protease gene and the first 300 amino acids of reverse transcriptase [5]. The PCR product was then analyzed with an ABI 3130 Genetic Analyzer (ABI, USA) using the chain termination method.

Drug resistance analysis

ContigExpress software (http://www.contigexpress.com) was used for sequence editing, assembly, and rectification. The resulting sequences were compared with the Stanford University HIV drug resistance database (http://hivdb.stanford.edu) version 6.1.1. for analysis of HIV drug-resistant mutations and drug resistance. A score >0 was regarded as drug resistance and >60 as high-level drug resistance.

Statistical analysis

The demographic characteristics of the patients were assessed using descriptive statistics. Continuous variables were reported as the mean and standard deviation (SD) and categorical variables were reported with frequencies and percentages. The risk factors for developing HIVDR were analyzed using multivariate logistic regression analysis, and odds ratios (OR) and 95% confidence intervals (CI) were obtained. For this analysis, the duration of first-line therapy was categorized as ≤36 months or >36 months, and the CD4 cell count was categorized as ≤200 cells/mm^3 or >200 cells/mm^3. All analyses were performed using SPSS 19.0 (IBM Corp., Armonk, NY, USA) and a two-sided P value <0.05 was considered statistically significant.

Ethics statement

This study was approved by the institutional review board of the First Hospital Affiliated to Henan University of Traditional Chinese Medicine. All of the individuals in this study were older than 18 years, and written informed consent was obtained before the study began.

Results

General information

A total of 122 patients met the enrolment criteria. Their mean age was 49.8 ± 8.5 years.

Table 1. Factors related with virologic failure and HIV drug resistance among HIV-infected patients receiving second line antiretroviral therapy

Variable	Cases	Virologic failure			HIV drug resistance		
		N (%)	OR (95% CI)	P	N (%)	OR (95% CI)	P
Total	122	33 (27.0)			18 (14.8)		
Sex							
M	71	16 (22.5)	1		8 (11.3)	1	
F	51	17 (33.3)	2.30 (0.90, 5.91)	0.083	10 (19.6)	3.32 (0.96, 11.50)	0.059
Age (years)							
≤50	68	16 (23.5)	1		7 (10.3)	1	
>50	54	17 (31.5)	1. 34 (0.51, 3.54)	0.556	11 (20.4)	1.80 (0.52, 6.29)	0.357
Married							
Yes	89	25 (28.1)	1		15 (16.9)	1	
No	33	8 (24.2)	0.68 (0.22, 2.07)	0.499	3 (9.1)	0.23 (0.04, 1.27)	0.093
Education level (years)							
≤6	76	23 (30.3)	1		12 (15.8)	1	
>6	46	10 (21.7)	0.86 (0.33, 2.27)	0.759	6 (13.0)	1.36 (0.39, 4.72)	0.633
Duration of first-line cART (months)							
≤36	46	19 (41.3)	1		9 (19.6)	1	
>36	76	14 (18.4)	0.33 (0.11, 1.00)	0.050	9 (11.8)	0.64 (0.15, 2.68)	0.536
Duration of second-line cART (months)							
≤36	55	11 (20.0)	1		7 (12.7)	1	
>36	67	22 (32.8)	1.41 (0.43, 4.64)	0.576	11 (16.4)	1.37 (0.30, 6.15)	0.685
Switch to second-line cART because of failed first-line cART							
Yes	88	29 (33.0)	1		17 (19.3)	1	
No	34	4 (11.8)	0.32 (0.09, 1.08)	0.067	1 (2.9)	0.13 (0.14, 1.25)	0.077
CD4 cell count (cells/mm^3)							
>200	103	2 2 (21.4)	1		10 (9.7)	1	
≤200	19	11 (57.9)	6.23 (1.86, 20.95)	0.003	8 (42.1)	10.62 (2.46, 45.91)	0.002

OR, odds ratio; CI, confidence interval; cART, combination antiretroviral therapy.

Seventy-one patients (58.2%) were men, 89 (73.0%) were married, 76 (62.3%) had achieved primary school education or less, all were farmers and of Han ethnicity, 110 (90.2%) were infected with HIV through plasma donation, and 89 (73.0%) were diagnosed with HIV infection between July 2003 and December 2004.

The first-line cART regimens included AZT+3TC+NVP (49.2%), AZT+3TC+EFV (8.2%), AZT+DDI+NVP (18.0%), AZT+DDI+EFV (2.5%), D4T+3TC+NVP (11.5%), and D4T+3TC+EFV (10.7%). All of the patients switched to 3TC+TDF+LPV/r as second-line therapy; 88 patients (72.1%) switched because of failed first-line therapy and others because of side effects. The mean durations of first-line cART and second-line cART were 67.1 ± 10.3 months and 27.6 ± 13.1 months, respectively.

Immunologic and virologic responses

At the study visit, the median CD4 cell count was 359 cells/mm^3 (interquartile range [IQR]: 255-554 cells/mm^3). The VL was <20 copies/mL for 47 of the 122 patients (38.5%), 20-1000 copies/mL for 42 patients (34.4%), and >1000 copies/mL for 33 patients (27.0%). Thus, 33 patients (27.0%) had virologic failure according to the 2012 revised World Health Organization recommendations, that defined the threshold of virologic failure as a concentration of >1000 copies/mL [11]. The median VL was 2.6 $\log_{10}$ copies/mL (IQR: 1.8-4.3 $\log_{10}$ copies/mL) for patients with detectable virus.

Prevalence of HIV drug resistant mutations

Genotypic resistance tests were performed on the samples of the 33 patients with a VL >1000

copies/mL, and protease and reverse transcriptase sequences were obtained. HIV was identified as HIV-1 subtype B in all samples. The prevalence of resistance to NRTI drugs was 9.8% (12/122), and the resistance-associated mutations were M184V (8/12), M41L (3/12), M42L (1/12), and K219E (1/12). The prevalence of resistance to NNRTI drugs was 11.5% (14/122), and the resistance-associated mutations were Y188L (6/14), V106I/A (6/14), V179D (5/14), K103N (3/14), Y181C (2/14), M230L (2/14), V108I (1/14), K101E (1/14), and G190A (1/14). Resistance to both NRTI and NNRTI drugs was present in 8 of the 33 samples (24.2%). Minor resistance mutations in the protease region were present in 15 samples; these mutations included A71T (10/15), A71V (4/15), and L10I (1/15).

Regarding the interpretation of drug resistance, HIV displayed resistance to 3TC in 36.4% (12/33) of samples, to AZT in 21.2% (7/33), D4T in 15.2% (5/33), DDI in 39.4% (13/33), TDF in 15.2% (5/33), EFV in 42.4% (14/33), and NVP in 42.4% (14/33) of samples. High-level drug resistance to 3TC, EFV, and NVP was found in 24.2% (8/33), 30.3% (10/33), and 39.4% (13/33) of samples, respectively.

Factors associated with virologic failure and HIV drug resistance

Based on the results of the multivariate univariate logistic regression analysis (**Table 1**), virologic failure was more common in patients with a shorter duration of first-line cART (OR 0.33; 95% CI 0.11, 1.00) and lower CD4 cell counts (OR 6.23; 95% CI 1.86, 20.95), whereas HIVDR was more common in those with higher CD4 cell counts (OR 10.62; 95% CI 2.46, 45.91).

Discussion

In this cross-sectional study, 33 (27.0%) of 122 HIV-infected patients on second-line cART experienced virologic failure, and 18 (14.8%) displayed HIVDR. A previously published systematic review reported that the occurrence of virologic failure varied considerably in different studies, ranging 8.6-37.3% at 6 months and 11.4-39.9% at 12 months, with most failures occurring within the first 6 months of initiation of second-line therapy [9]. Drug resistance occurred in 9.5% and 11.5% of participants at 12 months after initiation of second-line therapy in two cohort studies in China [10, 12]. These rates are lower than the rate observed in the present study. HIVDR did not occur in 45.5% (15/33) of the patients who experienced virologic failure in the present study; this could be partly explained by poor adherence, which most studies report to be the main reason for virologic failure [13]. In addition, the patients in the present study were from poor, rural areas, where medical resources may be limited and staff may have lower levels of education and less access to more advanced technology. Patients in these areas are significantly more likely to have HIVDR [14, 15]. In this study, the majority of patients were former plasma donors, and all were infected with HIV-1 subtype B strains, consistent with the results of other studies of former plasma donors [16].

The rates of HIVDR to NNRTIs and NRTIs were 42.4% and 36.4%, and the rate of HIVDR to both NRTIs and NNRTIs was 24.2% in the present study. Similar to other studies, M184V was the most common HIVDR mutation, followed by M41L, M42L, and K219E for NRTIs. M184V might alter the optimal binding of NRTIs and decrease 3TC removal and enzymatic process activity [17]. Although NNRTIs were not used in the second-line ART, NNRTI-related resistant mutations such as Y188L (6/14), V106I/A (6/14), V179D (5/14), K103N (3/14), and Y181C were still detected in some patients, similar to the results of other studies of second-line cART, confirming that these mutation sites might have little impact on viral replication ability [18]. There were some minor resistance mutations, such as A71T, A71V, and L10I in the protease region, but none of the samples displayed HIVDR to LPV/r, an important component of second-line cART.

The present results showed that HIVDR was associated with a switch to second-line cART because of failed first-line cART and lower CD4 cell counts. It is possible that drug resistance accumulated before the drugs were changed [12]. In a previous study, a longer duration of initial treatment was associated with more low-level drug resistance mutations, and increased the complexity of resistance genotypes [19], thereby reducing drug sensitivity. In addition, poor adherence might have contributed, because it is a significant risk factor for HIVDR. These results are similar to those obtained in other developing countries. Patients who failed

first-line treatment or who had no resistance likely had poor adherence to first-line therapy, whereas those with extensive resistance mutations at the time of virologic failure of first-line treatment are more likely to have poor adherence to second-line therapy [20, 21].

There were some limitations of this study. The cross-sectional design and the use of a single center limited the ability to detect clinical failures, which can occur during effective treatment [22]. Second, these results may not be fully representative of HIV-infected patients in China because of the small sample size and limited geographic distribution. Third, no data about the level of adherence were available in our study, which makes it difficult to compare our results with other studies in resource-limited settings.

In conclusion, the prevalence of HIVDR to second-line cART regimens was high. Although the drugs that are currently used are still effective, the accumulated resistant strains clearly show that emergence of HIVDR will continue to pose a challenge during the scaling up of treatment. Meanwhile, adherence to cART should be given greater attention.

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Disclosure of conflict of interest

None.

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