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HIV-1 Drug Resistance-Associated Mutations Among Antiretroviral-Naive Thai Patients With Chronic HIV-1 Infection

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Antiretroviral therapy (ART) has increased in resource-limited settings. This study determined the prevalence of HIV-1 drug resistance-associated mutations (DRAMs) among patients with chronic HIV-1 infections and compare DRAMs between CRF01_AE and B subtypes. ART-naive Thai patients who had ART initiation between 2010 and 2011 were enrolled prospectively. Genotypic assays were performed on viral reverse transcriptase and protease genes within 4 weeks before starting ART. DRAMs were assessed using the International AIDS Society-USA 2011 list. A total of 330 patients were included. HIV-1 subtypes included CRF01_AE (73%), B (23.9%), and others (3.1%). Median (IQR) CD4+ was 66 (23–172) cells/mm^3 and median (IQR) HIV-1 RNA was 5.2 (4.6–5.8) log copies/ml. The prevalence of patients with $\geq$1 DRAMs for any antiretroviral agents was 17.6%. DRAM prevalence was 17% for non-nucleoside reverse transcriptase inhibitors (NNRTIs), 0.6% for NRTIs, and 0.6% for protease inhibitors (PIs). DRAMs to NNRTIs were V106I (7%), V179D (4.2%), V179T (1.8%), E138A (1.5%), V90I (1.2%), K103N (0.9%), Y181C (0.9%), and P225H (0.3%). DRAMs to NRTIs were M184V (0.3%) and T215S (0.3%). The only major DRAM for PIs was M46L (0.6%). Minor DRAMs to PIs including I13V, M36I, H69K, and L89M were observed more frequently in CRF_01 AE. By multivariate analysis, the factors ''HIV-1 subtype B'' and ''low pretreated CD4+ cell count'' were associated with a higher rate of DRAMs. HIV-1 DRAMs, especially to NNRTIs, are emerging in a middle-income country after widespread use of NNRTI-based ART. HIV genotypic assays before ART initiation in patients with chronic HIV-1 infection should be considered. ***J. Med. Virol. 85:194–199, 2013.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: HIV; AIDS; primary resistance; mutations; Thailand

INTRODUCTION

The use of combined antiretroviral therapy (ART) has changed dramatically the course of human immunodeficiency virus type 1 (HIV-1) disease in patients who achieve durable virologic suppression, with a substantial reduction in morbidity, mortality, and HIV transmission [Palella et al., 1998; Manosuthi et al., 2006]. ART programs have been increasing in Southeast Asia for almost 10 years, commonly with a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen that is prescribed widely [Sungkanuparph et al., 2008]. In Thailand, the Ministry of Public Health established a national ART scaling-up program in 2002. However, emergence of HIV-1 drug resistance-associated mutations (DRAMs) after widespread drug use is a major contributing cause of treatment failure [Kuritzkes, 2004]. In addi-

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tion, response to initial therapy is compromised by pretreatment HIV-1 drug resistance. Transmission of HIV DRAMs and their effect on the response to first-line ART has been recognized for more than a decade in resource-rich countries [Little et al., 2002; Gallant et al., 2006]. However, little is known about HIV DRAMS in resource-limited countries. In addition, HIV drug-resistance testing before ART initiation has not been recommended routinely because of limited resources and a lack of data on primary HIV-1 drug resistance.

Circulating recombinant forms (CRF) of HIV-1 subtypes are common worldwide. Each CRF has a unique geographic distribution with the majority of CRF01_AE infections in Asia. Almost two million people live currently with HIV in South and Southeast Asia, and CRF01_AE is responsible for >80% of these infections [Hemelaar et al., 2006]. Therefore, we investigated the prevalence of primary HIV-1 resistance among antiretroviral drug-naïve patients with chronic HIV-1 infections who initiated ART. DRAMs in HIV-1 subtypes CRF01_AE and B subtypes were compared.

MATERIALS AND METHODS

All ART-naive patients infected with HIV who were indicated for ART initiation at the Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand were enrolled prospectively. This institute is a tertiary care and referral center for patients infected with HIV. The enrollment period was 2010 and 2011. Inclusion criteria were individuals infected with HIV who were (1) 18–60 years of age, (2) naïve to ART, and (3) had a CD4+ cell count <350 cells/mm^3. All patients received an ART regimen based upon the attending physicians' decision. The Institutional Ethics Committees of the Bamrasnaradura Infectious Diseases Institute and the Thai Ministry of Public Health approved this study, and all patients provided written, informed consent prior to enrollment. Demographic, clinical, and laboratory data were collected after receiving informed consent and within 4 weeks before ART initiation.

Blood samples were obtained to study CD4+ cell counts by flow cytometry using monoclonal antibodies with three colors reagent (TriTEST, Becton Dickinson BioSciences, San Jose, CA) and analyzed by FACScan flow cytometer (Becton Dickinson BioSciences). HIV-1 RNA viral load was evaluated by real-time PCR using a COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche Molecular Systems, Branchburg, NJ) that measures plasma HIV-1 RNA in a range of 40–10,000,000 copies/ml. Genotypic assays for viral reverse transcriptase and protease genes were performed within 4 weeks before ART initiation. HIV-1 RNA was isolated from plasma samples using a QIAamp viral extraction kit (Qiagen, Chatsworth, CA). The TRUGENE HIV-1 Genotyping Assay was used with an Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada) to sequence the protease and reverse transcriptase (RT) regions of the HIV-1 cDNA. This study focused on DRAMs at positions in the polymerase gene reported previously to be associated with antiretroviral drug resistance using the International AIDS Society-USA Drug Resistance Mutations 2011 criteria [Johnson et al., 2011]. Determination of HIV-1 subtypes was based on the genotypes of the reverse transcriptase and protease genes (TRUGENE HIV-1, Visible Genetics).

For this study, major DRAMS in the RT gene were considered to be M41L, K65R, D67N, insertion 69, K70R, L74V, Y115F, Q151M, M184V/I, L210W, T215Y/F, and K219Q/E for NRTI-associated mutations; and V90I L100I, K101E/P, K103N/S, V106A/M, V108I, E138A/G/K/Q/R, V179D/F/L/T, Y181C/I/V, Y188C/L/H, G190A/S, H221Y, P225H, F227C, and M230I/L for NNRTI-associated mutations. Major DRAMS in the protease gene were considered to be D30N, V32I, M46I/L, I47V/A, I50L, G48V, I50V, I54M/L, Q58E, T74P, V82A/F/S/T, N83D, I84V, N88D/S, and L90M. Complete blood counts, and tests for liver transaminases, serum creatinine, hepatitis B virus antigen (HBsAg), and antibody to hepatitis C virus (anti-HCV) were performed.

Mean (with standard deviation, SD), median (interquartile range at 25th and 75th, IQR 25th and 75th) and frequencies (%) were used to describe patient characteristics and parameters. Study patients were categorized into two groups according to HIV-1 subtype: CRF01_AE subtype or B subtype. Student's *t*-test was used to compare means of continuous variables with normal distribution between the two groups and Mann–Whitney *U*-test was used to compare medians of continuous variables with non-normal distribution. A chi-square test and Fisher exact test were used to compare categorical variables where appropriate. All analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL). A *P*-value less than 0.05 was considered statistically significant. Independent variables were evaluated with binary logistic regression to identify factors that were associated with DRAMs. By binary logistic regression analysis, any independent variable with a *P*-value of less than 0.1 was included in the multiple regression analysis model. Gender and HIV-1 subtype were examined as dichotomous variables and the percentage of CD4+ cell count was examined as a continuous variable.

RESULTS

This study had 330 patients with a mean age of 35.9 ± 9.1 years. Of these 236 (71.5%) were men, 329 (99.7%) were ethnic Thai, and 232 (70%) were heterosexuals. Median (IQR) CD4+ cell count was 66 (23–172) cells/mm^3 and median (IQR) plasma HIV-1 RNA was 5.2 (4.6–5.8) log copies/ml. Co-infection with hepatitis B virus was found in 15 patients (4.5%) and hepatitis C virus in 29 (8.7%). HIV-1 subtypes were CRF01_AE (241, 73.0%), B (79, 23.9%), and others (10, 3.1%). Table I compares demographic, clinical,

TABLE I. Demographics and Laboratory Data of Study Patients

Characteristics	Overall cohort (n = 330)	CRF01_AE subtype (n = 241)	B subtype (n = 79)	*P*-value
Demographics				
Male gender	236 (71.5%)	173 (71.8%)	56 (70.8%)	0.886
Age, years, mean ± SD	35.9 ± 9.1	36.1 ± 9.2	35.1 ± 8.5	0.410
Body weight, kg, mean ± SD	55.4 ± 11.0	55.1 ± 10.9	56.3 ± 11.7	0.403
Risk factor for HIV transmission				0.477
Sexual activity				
Heterosexual	212 (64.2%)	46 (66.0%)	46 (58.2%)	
Homosexual	91 (21.6%)	65 (27.0%)	25 (31.7%)	
Injection-drug use	6 (1.8%)	3 (1.2%)	3 (3.8%)	
Other/unknown	21 (6.4%)	14 (5.8%)	5 (6.3%)	
Laboratory parameters				
CD4 cell count, cells/mm^3, median (IQR)	66 (23–172)	54 (21–159)	94 (28–1,112)	0.076
Percentage of CD4 cell count, %, median (IQR)	7 (3–12)	6 (2–12)	9 (3–12)	0.044
Log plasma HIV-1 RNA, log copies/ml, median (IQR)	5.2 (4.6–5.8)	5.2 (4.6–5.8)	5.2 (4.6–5.8)	0.729
Hepatitis B virus antigen: positive	15 (4.5%)	12 (4.9%)	2 (2.5%)	0.003
Hepatitis C antibody: positive	29 (8.7%)	21 (8.7%)	5 (6.3%)	0.009

SD, standard deviation; IQR, interquartile range.

and laboratory data between CRF01_AE and B subtypes.

Table II shows HIV-1 DRAMs detected in the overall cohort and compares DRAMs between CRF01_AE and B subtypes. The prevalence of patients with ≥1 DRAMs to antiretroviral classes was 17.6%. This was classified as 17.0% to NNRTIs, 0.6% to NRTIs, and 0.6% to protease inhibitors (PIs). DRAMS for NNRTIs were V106I (23, 7%), V179D (14, 4.2%), V179T (6, 1.8%), E138A (5, 1.5%), V90I (4, 1.2%), K103N (3, 0.9%), Y181C (3, 0.9%), and P225H (1, 0.3%). DRAMS for NRTIs were M184V (1, 0.3%) and T215S (1, 0.3%). M46L (2, 0.6%) was the only major DRAM for PIs. Minor DRAMs to PIs included I13V, M36I, H69K, L89M, which were observed more frequently in CRF_01 AE subtype ($P < 0.05$), while A71I and V77I were observed more frequently in B subtypes ($P < 0.05$). Table III shows univariate and multivariate analysis of possible factors associated with any DRAMs after excluding minor DRAMs to PIs. By multivariate

TABLE II. HIV-1 Drug Resistance-Associated Mutations (DRAMs) Detected

DRAMs	Overall cohort (n = 330)	CRF01_AE subtype (n = 241)	B subtype (n = 79)	*P*-value
DRAMs to NNRTIs				
V106I	23 (7.0%)	16 (6.6%)	5 (6.3%)	1.000
V179D	14 (4.2%)	7 (2.9%)	7 (8.9%)	0.050
V179T	6 (1.8%)	4 (1.7%)	1 (1.3%)	1.000
E138A	5 (1.5%)	4 (1.7%)	1 (1.3%)	1.000
V90I	4 (1.2%)	4 (1.7%)	0 (0%)[a]	1.000
K103N	3 (0.9%)	2 (0.8%)	0 (0%)[a]	0.574
Y181C	3 (0.9%)	1 (0.4%)	2 (2.5%)	0.152
P225H	1 (0.3%)	0 (0%)[a]	1 (1.3%)	0.433
At least one NNRTI DRAM	56 (16.6%)	36 (14.5%)	16 (20.2%)	0.293
DRAMs to NRTIs				
M184V	1 (0.3%)	0 (0%)[a]	1 (1.3%)	0.433
T215S	1 (0.3%)	1 (0.4%)	0 (0%)[a]	0.433
At least one NRTI DRAM	2 (0.6%)	1 (0.4%)	1 (1.3%)	0.433
Major DRAMs to PIs				
M46L	2 (0.6%)	1 (0.4%)	1 (1.3%)	0.433
At least one PI DRAM	2 (0.6%)	1 (0.4%)	1 (1.3%)	0.433
Minor DRAMs to PIs[b]				
I13V	77 (23.3%)	75 (31.1%)	1 (1.3%)	<0.001
M36I	301 (91.2%)	234 (97.1%)	61 (77.2%)	<0.001
H69K	78 (23.6%)	77 (32.0%)	1 (1.3%)	<0.001
L89M	86 (26.1%)	85 (35.3%)	1 (1.3%)	<0.001
A71V	5 (1.5%)	0 (0%)[a]	4 (5.1%)	0.004
A71T	5 (1.5%)	1 (0.4%)	4 (5.1%)	0.004
V77I	3 (0.9%)	0 (0%)[a]	3 (3.8%)	0.048
Any DRAMs (excluding minor DRAMs to PIs)	58 (17.8%)	36 (14.5%)	18 (22.8%)	0.077

[a]Substitute 0 as 1 to calculate *P*-value.
[b]Only minor DRAMS to PI with a prevalence significantly different in CRF01_AE subtype and by B subtype are reported.

TABLE III. Univariate and Multivariate Analysis of Possible Factors Associated With DRAMs (Excluding Minor PI DRAMs)

	Univariate analysis			Multivariate analysis		
Parameters	*P*-value	OR	95% CI	*P*-value	OR	95% CI
HIV-1 subtype AE	0.040	0.535	0.294–0.972	0.025	0.498	0.270–0.917
Percentage of CD4+ cell	0.078	0.958	0.913–1.005	0.048	0.952	0.906–1.000
Female gender	0.081	1.698	0.936–3.077	0.086	1.699	0.928–3.110

OR, odds ratio; 95% CI, 95% confidence interval.

analysis, the factors "HIV-1 subtype B" and "low pretreated CD4+ cell count" were associated with a higher rate of DRAMs.

DISCUSSION

This study determined the prevalence of DRAMs among ART-naive patients with chronic HIV-1 infections who had ART initiation during 2010–2011. The results demonstrated that the majority of HIV-1 subtypes circulating were the CRF01_AE subtype. The HIV-1 subtype CRF01_AE was identified originally in Thailand in the early 1990s [McCutchan et al., 1992; Ou et al., 1993], and is the predominant infecting subtype in many parts of Asia, particularly in the southeast. Overall, DRAMs to any antiretroviral drug class were seen in 17.6% of study participants and the mutation at V106I in reverse transcriptase was found most frequently in this cohort. This proportion of DRAM is considered to be relatively high compared to previous studies conducted in the country and in other Southeast Asian countries, which had less than 7% [Apisarnthanarak et al., 2008; Sirivichayakul et al., 2008; Sungkanuparph et al., 2011, 2012; Le Nguyen et al., 2012]. This finding can be explained by the increased number of mutations listed in the International AIDS Society-USA Drug Resistance Mutations criteria [Johnson et al., 2011]. These include frequent DRAMs to etravirine, especially mutations V106I and V179D. Natural polymorphisms associated with etravirine resistance can occur in ART-naïve patients [Maiga et al., 2010]. Of note, these mutations have an impact on etravirine susceptibility as measured by weighted scoring: the etravirine-weighted score was 1.5 for each mutation. A previous study demonstrated that although the prevalence of etravirine DRAMs in ART-naïve patients infected with a non-B HIV-1 subtype was common, in most cases this had no significant impact on drug susceptibility [Maiga et al., 2010]. However, data on subsequent development of cumulative resistance upon receiving etravirine is still not available. Another explanation is that the DRAM interpretations are based upon different editions of drug resistance mutation lists. New DRAMs are added to the updated versions of mutation lists each year. In addition, the increased transmitted drug resistance seen in this study could be related to an increase in drug resistance among treated patients in the community. Finally, most previous studies were conducted among newly HIV-1 infected patients and this study was conducted in patients with chronic HIV-1 infection.

Primary HIV-1 drug resistance has been reported in Europe and North America and is 2–12% for NRTIs, 0–13% for NNRTIs, and 1–8% for PIs [2001; Grant et al., 2002; Little et al., 2002; Jayaraman et al., 2006; Oette et al., 2006; Booth et al., 2007]. Therefore, resistance mutation to NRTIs is the most frequent form of drug resistance, as found in previous studies. Recent studies those conducted in sub-Saharan Africa had reported a higher trend of primary HIV-1 drug resistance [Hamers et al., 2011; Hunt et al., 2012; Sigaloff et al., 2012; Somda et al., 2012]. For PI-associated mutations, the rate of resistance observed was lower than in previous reports from resource-rich areas. However, comparing the prevalence from different studies is difficult because many variables affect the estimates, including the time period of estimates, recent versus chronic HIV acquisition, definition of genotypic resistance, and risk populations. As expected, a number of polymorphism differences were seen in the protease gene between CRF01_AE and B subtypes. This study found that polymorphisms in the HIV-1 subtype CRF01_AE protease gene were common, with the M36I polymorphism the most frequent (91%). Although the M36I mutation is associated weakly with PI resistance when present with other DRAMs, M36I is a common non-subtype B polymorphism in the absence of drug pressure [Grossman et al., 2001; Holguin et al., 2002, 2006; Liu et al., 2007]. In the absence of drug pressure, a virus with the M36I polymorphism also has a higher replication capacity than wild-type virus in vitro [Holguin et al., 2006]. Thus, the presence of this polymorphism could provide a replicative advantage over the residue found naturally in subtype B. This suggests that 36I is most likely the natural genetic background of HIV-1 CRF_01 AE. In addition, host immune responses also drive genetic changes in HIV [Brumme et al., 2007]. Human leukocyte antigen alleles determine the cytotoxic T lymphocyte response, which targets specific HIV protein epitopes for virologic control [Ahlenstiel et al., 2007; Brumme et al., 2007; Ngumbela et al., 2008; Rousseau et al., 2008]. Variations in MHC class-I molecules among different human populations influence HIV evolution in a population [Ahlenstiel et al., 2007]. Therefore, further study is needed to examine the cytotoxic T lymphocyte-driven viral escape and the determinants of HIV evolution.

Association of CRF_01 AE with a lower probability of mutations in this study indicates a somewhat different pattern of sexual transmission between HIV-1 subtypes in Thailand and Asia [Ruxrungtham and Phanuphak, 2001; Ruxrungtham et al., 2004]. HIV-1 CRF_01AE was found mainly among heterosexual couples but subtype B was found among homosexual and bisexual men in Thailand [Ubolyam et al., 1994]. Most of the men who were infected with HIV subtype B had Western homosexual partners and homosexual and bisexual men tended to have higher rates of resistance than other groups [Ruxrungtham and Phanuphak, 2001]. This might explain why HIV-1 subtype B in this cohort had higher rate of DRAMs.

A number of study limitations should be acknowledged. Firstly, HIV-1 genotypic resistance testing was not performed on recently infected individuals. The reversion to a wild-type HIV-1 strain is possible, therefore, and the prevalence of detected primary resistance could diminish over time from transmission. Thus, the prevalence observed in this study might be an underestimate. Second, the results from this single healthcare center might not represent the entire country or region. Third, the sample size was relatively small. This factor might limit the ability to generalize to national prevalence. Further national and regional surveillance are still needed. Ultimately, mutations recognized in the World Health Organization (WHO) drug resistance mutations for surveillance 2009 list are based upon considerations that include any mutations that confer antiretroviral drug resistance, nonpolymorphic mutations, applications to most circulating subtype, and selection by drug pressure [Bennett et al., 2009]. Thus, the WHO mutations list is an appropriate tool for surveying transmitted HIV drug resistance, especially in patients infected newly with HIV, but not for monitoring HIV drug resistance in chronically infected patients. In chronic HIV infection, algorithms such as International AIDS Society-USA Drug Resistance Mutations list and the Stanford HIV resistance database are better for monitoring. In addition, these algorithms are based clinically and consider the effect of polymorphisms. Therefore, results derived from them provide information on the likely efficacy of current antiretroviral regimens in a country.

The acquisition of primary HIV-resistant strains is a main reason for failure of ART. Primary HIV-1 resistance, especially to NNRTIs, is emerging in a middle-income country after widespread use of NNRTI-based ART for a decade. An HIV genotypic assay before ART initiation in patients with chronic HIV-1 infection should be considered. In addition, some differences in DRAMs for PIs were observed between HIV subtypes.

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