Journal of Clinical Laboratory Analysis 27: 346–353 (2013)

Trends in Prevalence of HIV-1 Drug Resistance in Thailand 2009–2010

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Background: Treatment failure of antiretroviral therapy in HIV-1 infection is increasing due to development of viral resistance. Trends of resistance-associated mutation lead to the ineffective treatment in HIV-infected individuals. *Methods:* Extracted viral RNA from HIV-infected subjects in 2009 to 2010 was performed. The genotypic resistance testing was investigated for HIV-1 drug resistance in RT and PR genes. Frequencies of mutation were compared by a Fischer's exact test. *Results:* Three hundred and sixty-nine samples (147 in 2009 and 222 in 2010) were genotyped. At least one mutation was found in 90.8% (335/369) in PR gene and 87.0% (321/369) in RT gene. Three sequences in PR gene, M36I, H69K, and L90M, were decreased significantly in 2010 when compared to 2009. Mutations associated with resistance to nucleoside analogue reverse transcriptase inhibitors (NRTI's) were found in 61.0% and 64.2% in nonnucleoside analogue reverse transcriptase inhibitors (NNRTI's). A total of 49.6% was found in combined NRTI and NNRTI. In 2010, M41L was increased significantly from 7.5% to 14.9%. However, there was a decrease in the frequency of the mutations at position 67, 70, and 184 between 2009 and 2010. *Conclusions:* In 2010, three mutations in PR gene, M36I, H69K, and L90M, were decreased significantly. However, only one mutation in RT gene, M41L was significantly increased. J. Clin. Lab. Anal. 27:346–353, 2013.

Key words: HIV; prevalence; drug resistance

INTRODUCTION

The rapid development of resistance to single drug led to the use of combination therapies of three or more drugs. These combinations usually consist of a backbone of two complementary NRTI and either an NNRTI or one or two PIs. These combination therapies have been called highly active antiretroviral therapy (HAART). In terms of preventing the onset of resistance, this strategy has a number of advantages. First, the combination of drugs results in much greater levels of viral suppression; the reduction in viral turnover reduces the rate at which mutants are produced. Second, the development of resistance is much more complex as the virus must acquire mutations that induce resistance to a range of drugs, raising the genetic barrier (1, 2). However, virological failure of these regimens, due to development of viral resistance is becoming increasingly common (3–7). This indicates that current therapeutic regimens may not suppress virus sufficiently in the clinical situation to prevent development of resistance with selection of resistant quasi-species (2, 8, 9).

Factors leading to treatment failure in HIV-1 infection are numerous and complex. These are related to drug factors (limited potency of regimen, low genetic barrier, sub-inhibitory plasma levels, pharmacological factors), host-related factors (poor adherence, limited recovery

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Received 11 July 2012; Accepted 18 March 2013
DOI 10.1002/jcla.21609
Published online in Wiley Online Library (wileyonlinelibrary.com).

capacity of the immune system, prior drug experience), and viral-related factors (viral kinetics, error-prone reverse transcription, presence of resistant variants) (6, 10–21).

The capacity of HIV to develop drug resistance mutations is a major obstacle to effective long-term therapy (22–27). The increased use of combination antiretroviral therapies for HIV-1 infection has led to the emergence of viral strains resistant to all licensed reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs) (27–31). Mutations selected by drug resistance are classified as primary and secondary mutations. Primary mutations are generally selected early in the process of resistance mutation accumulation and tend to be specific for each compound. They have a marked effect on virus drug susceptibility in phenotypic assays. Secondary mutations by themselves have little or no discernible effect on resistance but may be selected because they compensate for reduced viral fitness induced by the primary mutation. Secondary mutations usually occur in viral genomes already containing one or more primary mutations. High prevalence of drug-resistant virus among HIV-1 infected patients on therapy has increased the probability of transmission of virus resistant to one or more classes of antiretroviral drugs (27–31). Complete suppression of HIV-1 could be compromised if therapy-naïve patients already harbor virus with mutations conferring resistance to antiretroviral drugs used for initial therapy (29,31,32). Furthermore, incomplete suppression of viral replication promotes the development of broader drug resistance, compromising subsequent treatment regimens. A number of studies have evaluated rates of resistance mutations in recently transmitted virus by looking at subjects with primary infection (28, 29, 32, 33). Reported rates of drug resistance in these studies vary from 1.4 to 37.0% with most of the resistance mutations seen in the RT gene (23,27,31,34–40). In this study, rates of genotypic resistance in both PR and RT genes were determined in HIV infection from 2009 to 2010 in Thailand.

MATERIALS AND METHODS

Patients and Blood Samples

The study was commenced in January 2009. All patients were identified with HIV infection and received antiretroviral therapy prior at the first plasma sample being collected. A sterile plasma sample stored at -70°C was included in the study.

Genotypic Resistance Analysis

Viral RNA and genotypic resistance testing from plasma was successfully determined on all subjects using standard methodologies. Plasma viral RNA was sequenced using commercially available kits. Briefly, the procedure was as follows: viral RNA was isolated from 140 μl plasma samples using the QIAamp Viral Extraction Kit (Qiagen Inc., Chatsworth, CA). The extracted viral RNA was reversed transcribed to cDNA using the RT-PCR Trugene kit. A 1.3 kb amplicon covering the entire polymerase gene, produced by single-tube PCR, was subjected to bidirectional DNA sequencing employing three primer pairs, and fluorescent dye primer chemistry on both forward and reverse strands (TruGene, Siemen). The sequencing reactions were loaded into an automated DNA sequencing system (CLIP, Siemen). This assay allows the sequencing of the amino acids 1–99 of PR and 1–247 of RT. Sequences were assembled and aligned with a lymphadenopathy-associated virus type 1 (LAV-1) consensus sequence using Trugene Gene Librarian Software. The software incorporates a rules based algorithm by comparing the derived patient sequence against the consensus to determine the presence of primary and secondary mutations. Genotypic resistance mutations were defined according to the International AIDS Society-USA recommendations (41).

Statistical Analysis

Comparison between frequencies of wild-type and mutant sequences were compared by a Fischer's exact test using Statistical Package for the Social Sciences (SPSS).

RESULTS

Between 1st January 2009 and 31st December 2010, 369 patients with HIV infection and appropriately stored plasma samples were identified. The demographic characteristics of this group are summarized in Table 1. Mean age was 39 years and the most common risk factor for acquisition of HIV-1 was sexual contact. These patients had high viral load (Table 1). Most of all viral sequences were A/E subtype (359/369) as determined using the Program Manual for the Wisconsin Package, Version 8 (Genetics Computer Group, Madison, Wisconsin) software. Ten of 369 were B subtype. Of these 369 patients, 147 presented

TABLE 1. Baseline Demographic Characteristics of 369 Subjects With HIV Infection

Age, mean (range)	39 (20–75)
Sex, number (%)	
Male	110 (29.8)
Female	101 (27.4)
MSM	158 (42.8)
Initial plasma HIV RNA level, (copies/ml) Mean (Range)	6350 (2,200–10,500)

TABLE 2. Frequency of Multiple Resistance Mutations in Single Isolate in 2009 and 2010

	Protease		Reverse transcriptase	
Number of mutations	2009	2010	2009	2010
1	22.0%	39.1%	19.5%	19.0%
2	29.3%	16.3%	9.8%	2.3%
3	4.9%	5.8%	2.4%	1.2%
4	0	1.2%	2.4%	0.4%
5	0	0.4%	0	0.4%

in 2009 and 222 presented in 2010. PR and RT sequences were obtained on all individuals. Table 2 summarizes the primary and secondary mutations detected in PR and RT genes in this population. At least one mutation associated with resistance to antiretroviral drugs was detected in 90.8% (335/369) in PR and 87.0% (321/369) in RT.

Resistance Mutations in PR Gene

Two hundred and sixty five (72.9%) sequences carried a primary protease inhibitor resistance mutation. Primary mutations observed were M46N/L, V82I, and L90M. All other resistance mutations were secondary mutations (Table 2). Among these primary mutations, L90M was found in 64.8%. In decreasing order of frequency, they were M36I, H69K, L10I/V, L63P/Q/N/T/A/S, K20R, V77I, and A71T/V. Figure 1 shows the number of primary and secondary mutations seen in PR each year. L90M was associated with H69K, while M46N/L and V82I were not accompanied by any secondary mutations. The most frequency of secondary mutations was found at position 36 (82.1%). If the data are considered, the most common polymorphism was M36I that occurred in 96.7% (142 of 147) in 2009 and 72.5% (161 of 222) in 2010. Three sequences in PR gene, M36I, H69K, and L90M, were decreased significantly in 2010 when compared to 2009 ($P < 0.05$). However, there was not significant increase in the proportion of PR resistance mutations represented by L101/V pre- and post-2009 from 23.13 to 26.1% (Fig. 1). If the data set is divided into sequences from patients presenting between 2009 and 2010 then 46.3% (68/147) and 48.2% (107/222), respectively of these groups carried sequences with at least one secondary mutation in the PR gene (Table 2).

Resistance Mutations in RT Gene

Mutations associated with resistance to nucleoside analogue reverse transcriptase inhibitors (NRTI's) were found in 61.0%, while those associated with resistance to nonnucleoside analogue reverse transcriptase inhibitors (NNRTI's) were found in 64.2%. Patients had combined NRTI and NNRTI mutations were found in 49.6%. Eleven different primary RT mutations were found: M184V (58.5%), Y181C/I (42.9%), K103N (21.0%), D67N (19.7%), G109S/A (19.7%), K70R (15.6%), K219Q/E (9.5%), M41L (7.5%), L210W (6.8%), T215Y/E (6.8%), and P225H (4.1%) in 2009 and M184V (53.1%), Y181C/I (35.1%), K103N (22.5%), G109S/A (19.4%), D67N (15.3%), M41L (14.9%), K70R (11.7%), K219Q/E (11.3%), L210W (6.8%), T215Y/E (9.9%), and P225H (2.7%) in 2010. Secondary mutations

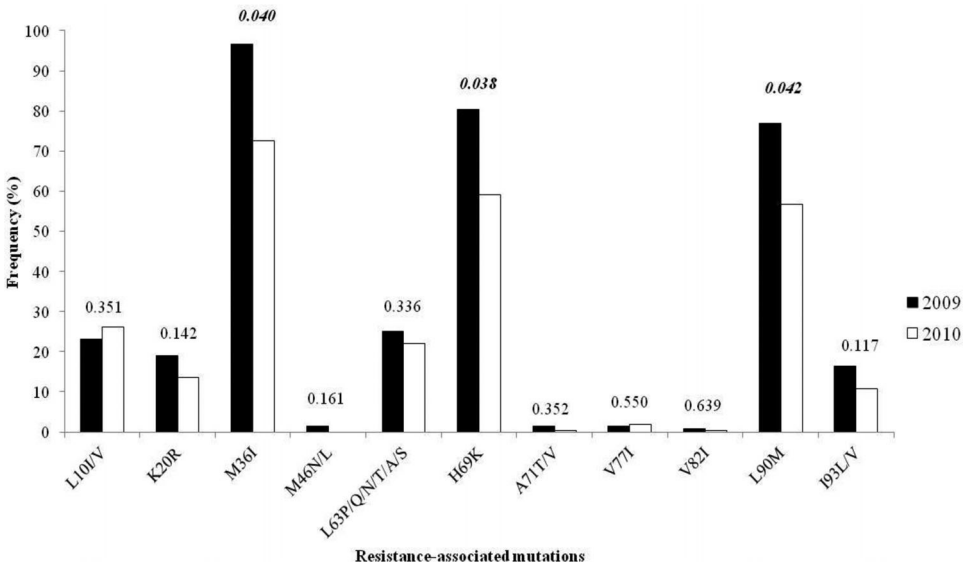

Fig. 1. Frequency histogram showing the rates of primary and secondary resistance mutations in the PR gene 2009 and 2010. *P* values are determined from a Fischer's exact test.

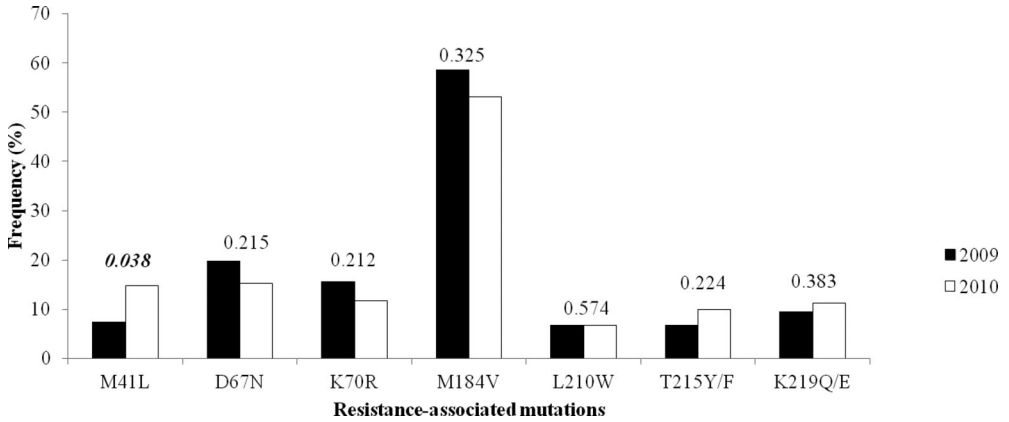

Fig. 2. There was AZT-related resistance mutation in RT pre- and post-2009. *P* values are determined from a Fischer's exact test.

in the RT gene were observed at positions 50 and 67. A total of 50.6% of subjects had one or two RT primary or secondary resistance mutations, 6.8% carried more than two mutations (Table 2). The frequency of primary and secondary mutations in the RT gene is shown in Figures 2 and 3. There was a decrease in the frequency of the following mutations: D67N (19.7 to 15.3%), K70R (15.6 to 11.7%), and M184V (58.5 to 53.2%) between 2009 and 2010 (Fig. 2) whereas the primary mutations at position 41, 215, and 219 were increased. In 2010, primary mutation in the RT gene at position 41 was increased from 7.5 to 14.9%. There was a statistically significant difference ($P < 0.05$). Of those, M184V was the most common (55.3%) that confers FTC or 3TC resistance. Combinations of this mutation are usually associated with high-level zidovudine resistance. Combined M184V and T215Y mutations were detected in 2.4% (9/369) and 12.7% (47/369) in 2009 and 2010, respectively. The resistance mutations of NNRTIs were detected in 64.2%. The most frequency of NNRTI resistance mutations were Y181C/I (38.2%), K103N (22.0%), and G190S/A (19.5%). The mutations at position 181, 188, 190, and 225 were decreased in 2010 when compared to 2009. In contrast to the mutation at position 103, there was an increase in 2010. However, it was not statistically significant difference ($P < 0.05$).

DISCUSSION

The development of resistance mutations in patients receiving combination therapy has resulted in resistant virus becoming more common within the HIV-infected population. It has raised concerns that such mutants may be transmitted more frequently, compromising not only the effectiveness of treatment of an individual but also the effectiveness of therapy on a population basis. Some reports have suggested that in excess of a quarter of subjects with recent acquisition of HIV have potentially drug-resistant virus (42–46).

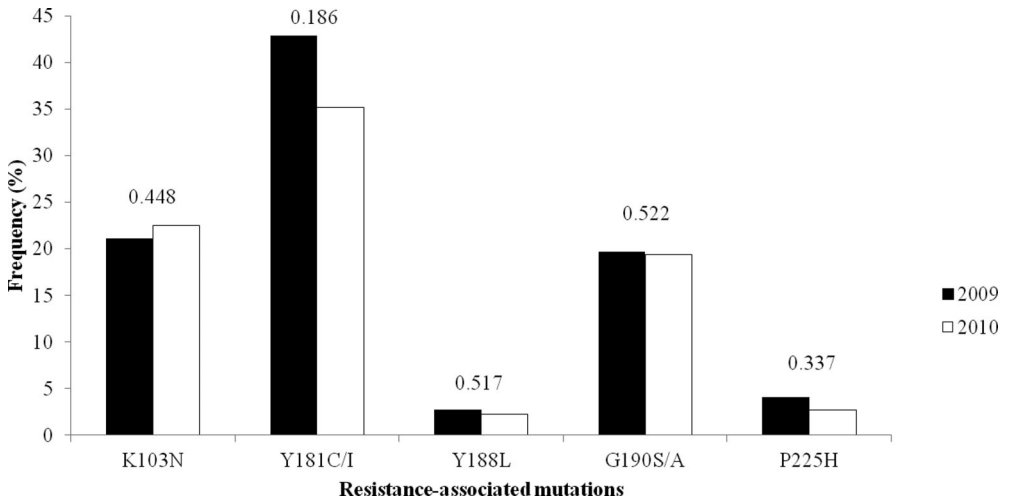

Fig. 3. There was NNRTI-related resistance mutation in RT pre- and post-2009. *P* values are determined from a Fischer exact test.

The increasing prevalence of drug-resistant mutations among HIV-1 infected patients on treatment has increased the probability of transmission of virus resistant to one or more classes of antiretroviral drugs (47–52). Incomplete suppression in turn promotes the development of broader drug resistance, compromising subsequent treatment regimens (1, 2, 6, 13).

Rates of new infection numbers caused by resistant virus vary markedly from study to study. Most studies report that at least 10% of new primary HIV-1 infected people carry virus resistant to at least one of the antiretroviral drugs while they are still therapy naive, suggesting that they have been infected with drug-resistant virus (53–66). However, some recent studies have suggested significant increases in the rates of transmission of drug-resistant HIV-1 within excess of 25% of newly infected individuals carrying virus with at least one primary mutation. (55, 67–72). By contrast, another European study sampling genotypes from 369 patients with recently acquired infection in Greece between 2000 and 2007 found that only 7.6% of viral sequences had mutations associated with NRTI resistance, 5.4% had mutations associated with NNRTI resistance, and only 3.3% had mutations associated with PI resistance (31).

Overall, most of the reported transmitted mutations occur in the RT rather than in the PR gene though relative frequencies vary markedly from study to study and may depend upon the dominant risk factors associated with transmission (31, 35, 45, 73–76). In general, higher rates of PR resistance have been seen in populations with intravenous drug use as a major risk factor, however the sample sizes employed in these studies are too small to make any firm conclusions regarding whether the types of transmitted resistance mutations is related to the mode of transmission (e.g. mucosal vs. blood borne transmission) (77–79).

While resistance mutations in RT are more commonly seen than mutations in PR; the RT mutations associated with AZT are the most commonly described (27,29,35,80,81). Relative rates of drug use are unlikely to explain the preferential finding of certain resistance mutations among resistant virus. It may be that the mutations most commonly reported in transmitted virus are those that have less implications in terms of viral fitness, especially in regards to transmission and initiation of infection (69, 82). There is a growing body of evidence supporting the phenomenon of reversion of mutations in the absence of drug pressure in transmitted virus, but reversion may not be to wild-type but to an intermediate strain. The best evidence for this phenomenon comes from the study of mutations at codon 215 in RT. The mutation T215Y is a common and well-described primary mutation for AZT resistance. In combination with M41L, it can induce high-level resistance to AZT. Although both these mutations are seen in transmitted virus, either alone or in combination, another set of mutations at 215 coding for one of the amino acids, D, C, or S have been found in a significant minority of samples (approximately 3–4%) in three independent studies (60, 69, 83, 84).

In our findings, frequency of mutations in PR gene has decreased in 2010. One of the reasons for the lack of increase in protease resistance mutations may be that the introduction of these drugs coincided with the institution of combination therapy. The resulting reductions in viral load and reduced rates of viral turnover resulting from these therapeutic strategies may have played a role in the lack of generation and transmission of protease inhibitor resistance.

Not only has the rate of mutations found in transmitted virus decreased but also the type of mutations seen also has changed, again reflecting changes in drug usage. The number of individual mutations associated with AZT resistance decreased, as did the number of combined AZT resistance mutations with decreased rates of AZT usage within the treated population. Notably, there was no significant increase in the rates of mutations associated with other RT inhibitors used within this population, which may reflect the increased use of these drugs in combination therapies during this period. This is especially noteworthy in the case of 3TC which is the most common NRTI included in the regimens of greater than 60% of those on treatment.

These data provide insight into how treatment strategies impact upon drug resistance virus. While the rates of transmission of resistance virus during the last decade are notable, they are much lower than those seen prior to the introduction of combination therapy. The change in rates of resistance seen in this study suggests that data derived from cross sectional studies should be interpreted with caution.

In this population, primary PR resistance mutations decreased and the rate of secondary resistance mutations was high since the introduction of protease inhibitors into this population. The prevalence of RT resistance mutations appears to be decreased. Only one mutation in this gene, M41L was significantly increased.

ACKNOWLEDGEMENT

This paper is supported by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

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