Resistance assays are useful in guiding decisions for patients experiencing virologic failure (VF) during highly-active antiretroviral therapy (HAART). We investigated antiretroviral resistance mutations in 41 Korean human immunodeficiency virus type 1 (HIV-1) infected patients with VF and observed immunologic/virologic response 6 months after HAART regimen change. Mean HAART duration prior to resistance assay was 45.3 ± 27.5 months and commonly prescribed HAART regimens were zidovudine/lamivudine/nelfinavir (22.0%) and zidovudine/lamivudine/efavirenz (19.5%). Forty patients (97.6%) revealed intermediate to high-level resistance to equal or more than 2 antiretroviral drugs among prescribed HAART regimen. M184V/I mutation was observed in 36 patients (87.7%) followed by T215Y/F (41.5%) and M46I/L (34%). Six months after resistance assay and HAART regimen change, median CD4+ T cell count increased from 168 cells/μL (interquartile range [IQR], 62-253) to 276 cells/μL (IQR, 153-381) and log viral load decreased from 4.65 copies/mL (IQR, 4.18-5.00) to 1.91 copies/mL (IQR, 1.10-3.60) (P<0.001 for both values). The number of patients who accomplished viral load <400 copies/mL was 26 (63.4%) at 6 months follow-up. In conclusion, many Korean HIV-1 infected patients with VF are harboring strains with multiple resistance mutations and immunologic/virologic parameters are improved significantly after genotypic resistance assay and HAART regimen change.

Key Words : HIV-1; Treatment Failure; Drug Resistance; Genotype

INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) in the treatment of human immunodeficiency virus type 1 (HIV-1) has led to marked improvement in morbidity and mortality associated with HIV-1 infection (1). However, treatment failure is inevitable in some patients with poor adherence, adverse effects, and acquisition of a resistance to antiretroviral drugs (2). Prospective data on resistance in patients with VF supports the concept that the virologic responses to therapy are better when the results of resistance testing are available compared to the responses observed when changes in therapy are guided only by clinical judgment only (3, 4). Various aspects of genotypic resistance and clinical application have been evaluated (5-9). However, some regional factors can affect the selection of treatment, such as the availability of specific antiretroviral drugs especially in heavily-treatment-experienced patients.

We believe it is worthwhile to reevaluate the benefits of genotypic resistance assay in various regional conditions, and this information might result in a better decision of treatment. Actually, transmitted drug resistant strains were reported in the Republic of Korea (10) as well as multi-drug resistant strains in treatment experienced patients (11, 12).

Since 2004, the Korea National Institute of Health has offered HIV-1 genotypic resistance assay to practicing hospitals. To assess the clinical influence obtained from resistance testing in this area, we analyzed genotypic mutations associated with antiretroviral resistance and observed virologic/immunologic parameters after genotypic resistance assay and HAART regimen change in Korean HIV-1 infected patients with virologic failure (VF).

MATERIALS AND METHODS

Study population and data collection

From August 2004 to July 2006, 80 patients were referred for genotypic resistance assay on account of VF. Among them, we analyzed virologic and immunologic response for HAART regimen change after genotypic resistance assay in 41 eligible patients with VF who satisfied following conditions: 1) Patients who were taking HAART for more than 6 months
when resistance assay was referred, 2) Patients with VF whose viral load assay results revealed log values of ≥ 3 more than twice during the 6 months prior to resistance assay referral, 3) Patients with available virologic and immunologic data for at least 6 months after resistance assay. Patients with a viral load of less than 1,000 copies/mL on resistance assay referral were excluded, for such cases were regarded not suitable for resistance assay because of result reliability concerns (13). Patients who did not change HAART regimen after resistance assay were also excluded from analysis (Fig. 1).

To comply with privacy concerns, a unique number system was adopted by the public healthcare system for psychosocial and financial support for people living with HIV/AIDS in the Republic of Korea. We obtained age, sex, antiretroviral treatment history, CD4+ T cell count, and viral loads in an anonymous data format from referring hospitals using this number system. The resistance referral format also contained information about the reason for resistance assay referral and adequacy of compliance.

Viral genotypic resistance assay

Viral genotypic resistance assay was performed with extracted viral RNA using silica-binding nucleic acid extraction (bioMérieux, Boxtel, The Netherlands). The polymerase chain reaction (PCR) amplification conditions were based on the Stanford Center for AIDS Research Laboratory protocol for sequencing the protease and reverse transcriptase genes. Sequencing reactions were performed using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Wellesley, MA, U.S.A.) on an automated sequencer (ABI Prism 3110 DNA sequencer). The pol gene sequences of eligible patients were submitted to Genbank as accession number EU290607-EU290646. Genotypic resistance mutation analysis was performed using the HIV Drug Resistance Database of Stanford University version 4.2.4 (http://hivdb.stanford.edu/).

Drug resistance determination

According to the HIV Drug Resistance Database of Stanford University, drug susceptibility was classified as ‘sensitive’ if the summed drug-specific scores were 0-9; ‘potential low-level resistance’ at 10-14; ‘low-level resistance’ at 15-29; ‘intermediate resistance’ at 30-59, and ‘high-level resistance’ at ≥ 60. To determine the susceptibility of a specific drug, we classified the drugs as ‘susceptible’ when the interpretation by the database was ‘sensitive’, ‘potential low-level resistance’, or ‘low-level resistance’. We considered the drugs as ‘resistant’ for interpretations of ‘intermediate resistance’ or ‘high-level resistance’.

Immunologic and virologic data analysis

The CD4+ T cell count and log viral load were compared between baseline and 6 months follow-up using a Wilcoxon signed rank test of paired tests to assess immunologic/virologic change after genotypic resistance assay and HAART regimen change in patients with VF. Statistical analysis was performed using SAS® version 9.1 (SAS Institute, Inc., Cary, NC, U.S.A.) and considered significant in cases of \( P<0.05 \).

RESULTS

Baseline clinical characteristics

Thirty-nine patients (95.1%) among 41 were male and the mean age was 41.9 ± 11.26 yr-old (Table 1). Most isolates observed were HIV-1 subtype B (38/41, 92.7%); 2 cases were isolates of subtype AG, and 1 was a subtype AG/G isolate. Mean HAART duration was 45.3 ± 27.5 months and the mean number of exposed HAART regimens before genotypic resistance assay referral was 2.22 ± 1.24. Suboptimal compliance was documented in 11 patients (26.8%) and these cases were associated with drug adverse reactions in most cases. The median baseline CD4+ T cell count and log viral load was 168 cells/μL (interquartile range [IQR], 62-253) and 4.65 copies/mL (IQR, 4.18-5.00), respectively.

HAART regimen and immunologic/virologic data after regimen change

When genotypic resistance assays were referred, 23 (56.1%)
Table 1. Characteristics and frequently observed resistance associated mutations of the 41 patients who changed HAART regimen after genotypic resistance assay

| Characteristics                      | Value                      |
|--------------------------------------|----------------------------|
| Mean age (yr)                        | 41.9 ± 11.26               |
| Male sex, number of patients (%)     | 39 (95.1)                  |
| Mean prior HAART duration (month)    | 45.3 ± 27.5                |
| Mean baseline CD4+ T cell count (cells/μL) | 195 ± 155               |
| Mean baseline log viral load (copies/ML) | 4.60 ± 0.72          |
| Mean 6 month follow up CD4+ T cell count (cells/μL) | 355 ± 223           |
| Mean 6 month follow up log viral load (copies/ML) | 2.03 ± 1.36        |
| Baseline HAART regimen before genotypic resistance assay, number of patients (%) | K219Q/E, 23 (56.1) |
|                                      | L210W, 6 (14.6)            |
|                                      | NRTI, 16 (39.0)            |
|                                      | K103N, 2 (4.9)             |
| New HAART regimen after genotypic resistance assay, number of patients (%) | V179D/E, 24 (58.5) |
|                                      | D30N, 12 (29.3)            |
|                                      | I54V, 4 (9.8)              |
|                                      | N88D, 1 (2.4)              |

*Frequently observed resistance associated mutations, number of patients (%)*

| Characteristics                      | Value                      |
|--------------------------------------|----------------------------|
| Antiretroviral resistance associated mutations (RT/PR)* | 11 (28.5) |
|                                      | 11.26 (11.26)             |
|                                      | 210W, 6 (14.6)            |
|                                      | 215Y/-, 6 (14.6)          |

(Continued to the next page)
patients were taking a protease inhibitor (PI) based regimen and 16 patients were taking a non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimen. Two patients were taking HAART regimen that included both PI and NNRTI. The most frequently prescribed HAART regimen was zidovudine (AZT)/lamivudine (3TC)/nelfinavir (NFV) (9/41, 22.0%) followed by AZT/3TC/efavirenz (EFV) (8/41, 19.5%), 3TC/stavudine (d4T)/EFV (4/41, 9.8%), 3TC/d4T/lopinavir/ritonavir (Kal) (3/41, 7.3%), and didanosine (ddI)/3TC/EFV (3/41, 7.3%) (Table 2). After genotypic resistance assay, 24 patients changed their HAART regimen to a PI based regimen, and NNRTI based regimen or PI+NNRTI based regimens were prescribed to 12 and 4 patients, respectively. AZT/3TC/Kal (9/41, 22.0%) was the most frequently prescribed regimen after genotypic resistance assay in VF patients. Six months after genotypic resistance assay and HAART regimen change, median CD4+ T cell count and log viral loads were 276 cells/µL (IQR, 153-381) and 1.91 copies/mL (IQR, 1.10-3.60) respectively, and both immunologic and virologic improvement were statistically significant compared to baseline values (P<0.001). Twenty-six patients (63.4%) accomplished a viral load <400 copies/mL 6 months after regimen change.

### Antiretroviral resistance associated mutations and susceptibility for HAART regimen

M184V/I mutation was observed in 36 patients (87.7%) followed by T215Y/F (17/41, 41.5%) and M46I/L (14/41, 34.2%). M46I, L54V, D30N, and V82A/T/S were observed in more than 10 patients and K103N (10/41, 24.4%) was the most frequently observed NNRTI associated mutation.
In regards to the susceptibility for respective drug included in HAART regimen with VF, almost all patients taking 3TC (36/37), EFV (18/18), NFV (12/12), and Kal (10/10) revealed intermediate to high level resistance while susceptibilities for AZT (11/20) and d4T (8/13) were preserved in more than half patients who were taking these drugs. Susceptibility for ddi was maintained in 3 among 9 patients (33.3%). Regarding to the number of susceptible drugs among HAART regimen prior to resistance assay referral, 40 patients (97.6%) were taking 1 or no susceptible antiretroviral drugs before genotypic assay and all prescribed antiretroviral drugs were non-susceptible in 18 patients (43.9%). After resistance assay, 2 or more antiretroviral drugs in new HAART regimen were susceptible in 31 patients (75.6%).

**DISCUSSION**

The genotypic resistance assay has become the standard of care in the treatment of HIV-1 infected patients especially in cases of VF, and its efficacy has been proven by numerous studies as well as the phenotypic resistance assay. Although Luca et al. (14) observed variations in the prediction of subsequent antiretroviral treatment outcomes among different genotypic resistance interpretation systems of rules-based algorithms, we believe that these systems will evolve through the accumulation of data from treatment experience. During our study period, PI based regimens comprised 56.1% (23/41) of patients with VF and NFV was the most commonly prescribed PI (12/23, 52.2%). Among these 23 patients with a PI based regimen, 12 patients (52.2%) changed to a NNRTI based regimen while 6 patients (26.1%) maintained PI based regimens substituted with newer PIs. However, all 16 patients that failed the NNRTI based regimen changed to a PI based regimen. This implies that invariable cross resistance among NNRTIs affected HAART regimen selection in these patients.

The most frequently prescribed nucleoside reverse transcriptase inhibitor (NRTI) backbone in VF patients was AZT/3TC (20/41, 48.8%). During our study period, available NRTIs in the Republic of Korea were AZT, 3TC, d4T, ddi and abacavir. Almost all patients were taking 3TC in our study group and most isolates of these patients revealed resistance to 3TC associated with M184V/I mutation. It is known that presence of the M184V mutation might delay or prevent emergence of thymidine analogue mutations (TAM) (15, 16). In our study group, 31 patients were prescribed with thymidine analogues (AZT or d4T) with 3TC. Among them, all revealed intermediate to high-level resistance to 3TC while only 10 patients (32.3%) revealed non-susceptibility to thymidine analogues and this phenomenon was observed in both PI based regimens (5/19) and NNRTI based regimens (5/10). Except for thymidine analogues and ddi, most antiretroviral drugs involved in failing HAART regimens proved to be non-susceptible as described above. Considering that tenofovir is not available and a combination of ddi and d4T is not recommended on account of toxicity (13), optimizing the NRTI backbone might not be feasible in patients with VF. Furthermore, AZT is frequently complicated with bone marrow suppression.

Six months after genotypic resistance assay and HAART regimen change, CD4+ T cell count and log viral load improved significantly. While 40 patients were taking 1 or no susceptible antiretroviral drugs before genotypic assay, 31 patients (75.6%) were prescribed new regimens containing 2 or more susceptible antiretroviral drugs after resistance assay. However, the response for HAART is influenced by many other factors such as drug compliance, as well as resistance. Our study included 11 patients with suboptimal compliance before genotypic resistance assay and most cases were associated with drug adverse effects. The immunologic and virologic improvement observed in our study group might be associated with a compliance change. However, neither baseline immunologic/virologic parameters nor improvement of them 6 months after HAART regimen change were different according to drug compliance before resistance assay (non-parametric analysis, data not shown). This indirectly implies that the influence of compliance on immunologic/virologic improvement after resistance assay was limited in our study group. Furthermore, suboptimal compliance is one of the crucial conditions for antiretroviral resistance acquisition and resistance assay might be quite beneficial for these patients (17). In our study group, 26 patients (63.4%) accomplished viral load <400 copies/mL 6 months after regimen change and it is comparable with that of antiretroviral naïve patients starting HAART (103/141, 73%) as previously reported in the same country (18). We supposed that our study group revealed fair results considering that almost all patients harbored strains with resistance to 2 or more classes of antiretroviral drugs.

As we described before, 7 patients with VF did not change their HAART regimens after genotypic resistance assay (Table 2). This group consisted of 2 patients with multiple resistances and 5 patients with limited resistance profiles. Case 46, who was taking AZT/3TC/Kal, revealed a high-level resistance to all antiretroviral drugs analyzed by Stanford database (version 4.2.4). Case 42, who was taking ddi/Kal/EFV, also revealed a high-level resistance to all antiretroviral drugs except for AZT, d4T, and tenofovir which was not available during our study period. However, the patient had been not tolerated AZT before resistance assay referral because of neutropenia and d4T was included in the prior VF HAART regimen. The CD4+ T cell count and viral load aggravated in these patients after maintenance of the same HAART regimen for 6 months. For the other 5 patients, equal or less than one antiretroviral drug revealed resistance among prescribed HAART regimen. In these patients, the CD4+ T cell count/viral load revealed various progresses after maintenance of same HAART
regimen for 6 months. In a comparison of the HAART regimen change group and HAART regimen non-change group after resistance assay, increases in both the median CD4+ T cell count and decreases in median log viral load were more favorable in the HAART regimen change group (P=0.019 and P<0.001, respectively. Wicoxon rank sum test, data not shown). We supposed this was partially due to the characteristics of the study groups as well as HAART regimen change. However, it may not dwindle the efficacy of genotypic resistance assay in VF. A randomized study comparing HAART regimen change with resistance assay results and HAART regimen change with only prior HAART history and virological/immunological responses would prove the efficacy of resistance assay more effectively, but it was not possible to analyze our data in this manner.

This study has a few limitations. First, this study includes a small number of patients and only short term follow-up data were available. However, this study will help to identify resistance patterns and clinical responses after genotypic resistance assay in Korean HIV-1 infected patients with VF. Second, patients with suboptimal compliance were included in analysis and this factor might have confounded the benefit of resistance testing, although it was not significant as mentioned above. However, considering the close relationship between suboptimal compliance and antiretroviral resistance acquisition, we believe that it might warrant vigorous resistance testing for these patients as well as patients with fair compliance.

In conclusion, many Korean HIV-1 infected patients with VF are harboring strains with multiple genotypic resistance mutations and immunologic/virologic parameters are improved significantly after genotypic resistance assay and HAART regimen change.

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