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Low Prevalence of Drug-Resistant HIV-1 in Patients Newly Diagnosed with Early Stage of HIV Infection in Korea

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There is a concern that the widespread use of antiretroviral drugs to treat HIV infections may result in the increased transmission of a drug-resistant virus. Drug resistance testing before initiating treatment among newly diagnosed HIV patients is helpful in the design of initial regimens. Although HIV infected patients have been increasing in Korea, the transmission rate of drug-resistant HIV is unknown. The aim of this study was to determine the prevalence of antiretroviral drug resistance-associated mutations in patients newly diagnosed as early stage of HIV infection in Korea. We defined patients with early HIV infections as those with confirmed diagnoses who had an indeterminate Western blot. We performed genotypic resistance testing in 66 HIV-1 subjects at an early HIV infection stage who were identified between March 2002 and June 2005. Two of the 66 subjects with early HIV infections showed major mutations associated with resistance. Major mutations by themselves reduce susceptibility to one or more drugs and occur commonly during virological failure. Minor mutations have little or no effect on susceptibility and occur only after other drug-resistance mutations. The resistant mutation of reverse-transcriptase gene was found at E44D, and the major resistant mutation of protease gene was found at M46L. Minor protease resistance mutations were seen in 52 cases. Genetic subtype analysis revealed that all subjects were infected with HIV-1 subtype B. In conclusion, the prevalence of drug-resistant HIV-1 in patients newly diagnosed with HIV in its early infection stage is not high in Korea. ——— anti-retroviral drugs; genotypic drug resistance; HIV; HIV infection; resistance mutation.
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The use of intensive antiretroviral therapy has resulted directly in dramatic declines in morbidity and mortality among HIV-infected patients and often in substantial recovery of impaired immunologic function (Autran et al. 1997; Palella et al. 1998). Resistance to antiretroviral drugs has

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often developed in patients with incomplete viral suppression and may limit both the magnitude and duration of the response to treatment (Little et al. 2002). Resistance testing can help identify more-effective antiretroviral regimens for patients for whom therapy is failing and has been incorporated into clinical treatment guidelines (Sax et al. 2005).

Increases in the prevalence of drug-resistant viruses among HIV-infected patients have been associated with an increase in drug-resistant virus transmission to newly-infected patients. The efficacy of initial antiretroviral treatment is limited by the transmission of drug-resistant HIV strains (Little et al. 2002). In addition, the rate at which transmitted resistance-associated mutations revert to wild type has not been completely delineated, but mutations present at the time of HIV transmission are more stable than those selected under drug pressure (Novak et al. 2005). Therefore, performing genotypic resistance testing before treating a newly HIV-infected patient with antiretroviral drugs can increase the anti-retroviral drug therapy response rate (Boden et al. 1999; Durant et al. 1999).

In Korea, all cases of HIV infection are confirmed by the Division of AIDS at the Korea National Institute of Health (KNIH). Recently, the number of persons with indeterminate HIV-1 Western blot results has increased in Korea. Most of these persons with indeterminate HIV-1 Western blot assays were confirmed to have HIV infections based on a follow-up HIV-1 Western blot assay. We therefore regarded these cases as newly diagnosed HIV-1 patients in the early stage of HIV infection (Little et al. 2002). We designed our study to determine the prevalence of antiretroviral drug-resistant mutations in Koreans with HIV in the early stage of infection.

Material and Methods

Study subjects and design

In Korea, 2,222 patients infected with HIV were reported between 2002 and 2005. During this same period, 86 HIV-infected patients in the early HIV infection stage were confirmed by the following methods in the Division of AIDS at the Korea National Institute of Health (KNIH). In our study, patients with early HIV infections were defined using a detectable HIV enzyme immunoassay (EIA; Vironostika HIV-Uni-Form II Plus O; bioMérieux, Boxtel, The Netherlands) and an indeterminate result in the HIV-1 Western blot assay (HIV Blot 2.2, Genelabs Diagnostics, Singapore) which documents early HIV infection (Little et al. 2002; Zhong et al. 2003). Early HIV-1 infection was confirmed by a later HIV-1 Western blot assay and an HIV-1 RNA quantification test (NucliSens HIV-1 RNA QT bioMérieux, Inc. [formerly Oreganon Teknika], Durham, NC, USA) (Brambilla et al. 2003). Twenty subjects among the patients with early HIV infections did not complete the drug resistance test because of inadequate sample volume and low HIV-RNA copies. We enrolled a total of 66 subjects in our study. Their epidemiological records were reviewed retrospectively. These records included medical records from the first diagnostic exam and all clinical follow-up exams. The samples which yielded indeterminate Western blot results were used for genotypic resistance testing.

Genotypic resistance testing

Viral genotypic resistance testing was performed on extracted viral RNA using the QIAamp viral nucleic acid extraction kit (Qiagen, Valencia, CA, USA) (Boden et al. 1999). 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.

Sequence analysis

Sequences were determined using an ABI Prim Dye Terminator Cycle Sequencing Ready Reaction Kit (PerkinElmer, Wellesley, MA, USA) and an automated sequencer (ABI Prism 3110 DNA sequencer). The drug-resistant results were analyzed according to the Stanford HIV-DB and International AIDS Society-USA Drug Resistance guidelines (Johnson et al. 2008). The genetic subtype was determined by a sequence analysis program (http://hivdb.stanford.edu/). The study protocol was approved by the ethics committee of the Korea National Institute of Health and Yonsei University College of Medicine.

Results

We analyzed the data from 66 patients with early stage HIV-1 infections. A total of 62 (93.9%) of the subjects were men, and the mean age at the

TABLE 1. Demographic characteristics of patients with early HIV-1 infections at diagnosis (n = 66).

Characteristic	Value
Sex – No. (%)	
Male	62 (93.9)
Female	4 (6.1)
Age – yr	
Mean (range)	36.0 (15-75)
Route of transmission - No. (%)	
Heterosexual contact	32 (48.5)
Homosexual contact	31 (46.9)
Unknown	3 (4.6)
Symptomatic HIV-1 patients	
/asymptomatic HIV-1 patients - No. (%)	48/18 (72.7/27.3)
Symptoms in HIV-1 patients - No. (%)	
Fever	42 (87.5)
Fatigue	20 (41.7)
Weight loss	13 (27.1)
Lymphadenopathy	11 (22.9)
Myalgia or arthralgia	9 (18.8)
Cough	8 (16.7)
Diarrhea	6 (12.5)
Elapsed time - days	
From indeterminate to confirmed Western blot	
Median (range)	10.5 (3-46)
CD4+ T lymphocyte count - cells/mm^3 (n = 58)	
Median (range)	397.5 (33-981)
Plasma HIV RNA - log copies/ml (n = 66)	
Average (range)	6.10 (2.79-7.26)

time of the HIV-1 diagnosis was 36.0 ± 10.9 years. All enrolled subjects were infected in Korea according to epidemiological records. Among HIV-1 patients in the early stage of HIV infection, 42 (87.5%) were identified as having a symptomatic primary HIV infection. The elapsed time from the initial indeterminate Western blot assay to the confirmed Western blot assay was 10.5 days. The median CD4+ T cell count at diagnosis was 397.5 cells/mm^3, and the mean plasma HIV-RNA levels were 6.10 log copies/ml (Table 1). Of the 66 subjects, there were two HIV-1 persons with drug resistance-associated major mutations. These two HIV-1 patients were both men, one of whom reported heterosexual behavior and the other reported homosexual behavior. One of the 66 subjects had a nucleotide reverse transcriptase inhibitor (NRTI)-related genotypic mutation at E44D. The other subject had a protease inhibitor (PI)-related major genotypic mutation at M46L. Neither subject had non-nucleotide reverse transcriptase inhibitor (NNRTI)-related genotypic mutations. Fifty-two (78.8%) persons had protease inhibitor (PI)-related minor genotypic mutations: L10I/V, K20R/I, M36I/L, D60E, L63P, A71V/T, V77I, and I93L (Table 2). All subjects were infected with HIV-1 subtype B.

TABLE 2. Distribution of resistance-associated mutations in patients with early HIV-1 infection.

	NRTI*	NNRTI†	PI‡	
			major resistance	minor resistance
No. 1	-	-	-	M36I, I93L
No. 2	-	-	-	V77I
No. 3	-	-	-	M36I
No. 4	-	-	-	L10I, M36I
No. 5	-	-	-	-
No. 6	-	-	-	M36I, L63P, V77I, I93L
No. 7	-	-	-	L10I, I93L
No. 8	-	-	-	-
No. 9	-	-	-	M36L
No. 10	-	-	-	L10I, V77I
No. 11	-	-	-	V77I
No. 12	-	-	-	I93L
No. 13	-	-	-	V77I, I93L
No. 14	-	-	-	L10I, V77I, I93L
No. 15	-	-	-	V77I, I93L
No. 16	-	-	-	V77I-
No. 17	-	-	-	V77I
No. 18	-	-	-	L10I, I93L
No. 19	-	-	-	L10I, V77I, I93L
No. 20	-	-	-	L10V, K20I, M36I, L63P
No. 21	-	-	-	-
No. 22	-	-	-	I93L
No. 23	E44D	-	-	V77I
No. 24	-	-	-	V77I
No. 25	-	-	M46L	V77I, I93L
No. 26	-	-	-	A71V, V77I, I93L
No. 27	-	-	-	L10I, V77I
No. 28	-	-	-	L10I. M36I, I93L
No. 29	-	-	-	K20R
No. 30	-	-	-	V77I
No. 31	-	-	-	-
No. 32	-	-	-	L10I, I93L
No. 33	-	-	-	L10I, I93L
No. 34	-	-	-	-
No. 35	-	-	-	-
No. 36	-	-	-	L10V, K20I, M36I, L63P
No. 37	-	-	-	M36I, L63P
No. 38	-	-	-	-
No. 39	-	-	-	V77I
No. 40	-	-	-	A71T, V77I, I93L

TABLE 2. Continued

	NRTI*	NNRTI†	PI‡	
			major resistance	minor resistance
No. 42	-	-	-	A71T, V77I, I93L
No. 43	-	-	-	L10I, I93L
No. 44	-	-	-	V77I
No. 45	-	-	-	L10V, M36I
No. 46	-	-	-	-
No. 47	-	-	-	M36L
No. 48	-	-	-	V77I
No. 49	-	-	-	-
No. 50	-	-	-	M36L
No. 51	-	-	-	-
No. 52	-	-	-	L10I, L63P
No. 53	-	-	-	M36I
No. 54	-	-	-	L10I, L63P
No. 55	-	-	-	L10I, I93L
No. 56	-	-	-	V77I
No. 57	-	-	-	-
No. 58	-	-	-	-
No. 59	-	-	-	L10I, D60E
No. 60	-	-	-	-
No. 61	-	-	-	-
No. 62	-	-	-	M36L
No. 63	-	-	-	L10I, I93L
No. 64	-	-	-	M36I
No. 65	-	-	-	V77I
No. 66	-	-	-	L10I, L63P

*Nucleotide reverse transcriptase inhibitor ‡Protease inhibitor
†Non-nucleotide reverse transcriptase inhibitor

DISCUSSION

According to UNAIDS, the estimated prevalence rate of HIV positivity in Korea is less than 0.1%. As of December 2006, 4580 HIV-infected patients were officially identified in Korea (Oh et al. 2007b). Although there is not yet a high prevalence rate of HIV-infection in Korea, the incidence of new diagnoses of HIV-infected patients is increasing. In 2002, the number of newly-identified HIV-infected patients was 398, whereas in 2006 this figure increased to 751.

Since HIV has high mutation rate and patients with HIV need lifelong treatment, there is a high probability that HIV drug resistance will emerge in regions where antiretroviral therapy is rapidly being scaled-up, just as it has already emerged in developed countries. In Korea, zidovudine has been supplied by the government without charge since 1990 through a nationwide AIDS program which has resulted in easy access to long-term monotherapy for HIV-1 patients (Park et al. 2003). In 1998, the protease inhibitor (PI) indinavir was first introduced into practice. It is known that over 44 medical institutions in Korea have currently used more than 10 drugs

(indinavir, nelfinavir, ritonavir/lopinavir, lamivudine, zidovudine, stavudine, zalcitabine, didanosine, efavirenz, nevirapine, abacavir, atazanavir) for HAART regimens. According to Korean research data, the proportion of HIV-infected patients receiving antiretroviral drug therapy is about 66.1% (Kim et al. 2007). Initial antiretroviral drug therapy regimens were NNRTI-based regimens (14.2%) and PI-based regimens (85.8%). Indinavir is the most commonly-used PI-based drug (66.0%); the second most popular drug is lopinavir/ritonavir (17.7%).

However, the frequency of antiretroviral drug resistance in a treatment-failure group ranged between 42%-71% (Chang 2002). M41L, K70R, L74V, V118I, M184V/I, T215Y/F, and K219Q/E were frequent NRTI-related genotypic mutations with a frequency of over 10%, and L100I and K103N were the most frequently mutations seen in NNRTI. PI-related major genotypic mutations were D30N, M46I/L,G48V, I50V, V82A/F/T/S, I84V, and L90M in 4.4%-24.4% of drug-resistant patients (Oh et al. 2007a). Additionally, it is known that the drug resistance rate of drug-naive HIV-1 group is as low as 2.3%-14% (Kim et al. 2001; Chang 2002; Park et al. 2003). However, the level of drug resistance in acute or early stages of HIV infection had not yet been determined in Korea prior to this study.

According to recently standardized surveillance reports of HIV-1 drug resistance, commonly recognized drug resistance mutations were excluded from the list because of occurrence above the cut-off in at least one subtype with no evidence of exposure to drug pressure (Shafer et al. 2007). M46L has been reported in 1.5% (4/264) of subtype G sequences and E44D has been reported in 1.6%, 1.5%, and 0.7% of persons with subtypes D, F, and G, respectively (Shafer et al. 2007). Therefore, our mutation results might represent normal polymorphisms.

We examined only a small subject group and our results might therefore be an underestimation of the frequency of disease-resistant HIV strains in newly infected persons. The prevalence of drug resistance we detected was lower than that identified in the United States and Europe (Balotta et al. 2000; Yerly et al. 2001). These studies have identified drug resistant variants in 22.7% of patients newly-infected with HIV in the United States and 9%-21% of patients newly-infected with HIV in Europe. Although the reason for the low prevalence rate of drug resistance in Korea is unclear, it may derive from differences in the test methods used to determine drug resistance, geographic variability in patterns of antiretroviral-drug use, sexual culture, and risk factors for HIV exposure (Little et al. 2002). Furthermore, it is known that injectable drug use is a risk factor for poor adherence to antiretroviral treatment, a behavior which results in the development of drug-resistant HIV (Moss et al. 2004; Gatanaga et al. 2007). The low frequency of drug-resistant transmission in the Korean population could be due to the fact that the frequency of injection drug users (0.1%) is lower among HIV/AIDS patients in Korea (Lee et al. 2008). Another possible explanation is the system that manages HIV-infected patients in Korea. All KNIH-confirmed HIV infected cases are registered and these patients undergo periodic follow-ups that include counseling, physical and immunological status checks, health education, and an assessment of the patient's need for economic support by the governmental management system (Shin et al. 1998). We suggest that governmental health checkups at regular intervals for HIV-infected patients may contribute towards the low transmission rate of drug-resistant HIV in Korea.

Based on our study results, we suggest that baseline genotypic resistance testing of all infected patients would be cost-effective for guiding initial therapy in Korea (Weinstein et al. 2001; Hammer et al. 2006). Furthermore, according to a recent study, resistance testing that is performed at the time of HIV diagnosis with a baseline resistance prevalence of >1% is a cost-effective strategy that can lead to the selection of a more effective initial antiretroviral regimen and likely a longer survival for patients who have been infected with drug-resistant viruses (Sax et al. 2005).

In Korea, antiretroviral drug-resistance testing is not yet widely performed. Although our study results may not be of major clinical impor-

tance, our findings indicate the necessity of establishing a diagnostic system for newly HIV-infected persons and continuing drug-resistance surveillance studies according to the World Health Organization protocol.

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