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Trends in transmitted drug-resistant HIV-1 and demographic characteristics of newly diagnosed patients: Nationwide surveillance from 2003 to 2008 in Japan

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ABSTRACT

The emergence and transmission of drug-resistant human immunodeficiency virus-1 (HIV-1) compromises antiretroviral treatment for HIV-1. Thus, testing for drug resistance is recommended at diagnosis and before initiating highly active antiretroviral treatment. We conducted an epidemiological study enrolling newly diagnosed patients between 2003 and 2008 in our nationwide surveillance network. In the 6-year study period, the prevalence of drug-resistant HIV-1 among 2573 patients, consisting mainly of Japanese men in their late-30s and infected through male-to-male sexual contacts, followed an increasing trend from 5.9% (16/273) in 2003 to 8.3% (50/605) in 2008. Nucleoside reverse transcriptase inhibitor-associated mutations predominated in each year, with T215 revertants being the most abundant. The predictive factor for drug-resistant HIV-1 transmission was subtype B (OR = 2.36; $p = 0.004$), and those for recent HIV-1 infection were male gender (OR = 3.79; $p = 0.009$), MSM behavior (OR = 1.67; $p = 0.01$), Japanese nationality (OR = 2.31; $p = 0.008$), and subtype B (OR = 5.64; $p < 0.05$). Continued activities are needed to raise awareness of the risks of HIV-1 infection and complications of drug-resistant strains. Continued surveillance is also needed to understand trends in the HIV-1 epidemic.

Abbreviations: HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; PI, protease inhibitor; HBV, hepatitis B virus; HCV, hepatitis C virus; PR, protease; RT, reverse transcriptase; RT-PCR, reverse transcription polymerase chain reaction; CRF, circulating recombinant form; NRTI, nucleoside RT inhibitor; NNRTI, non-nucleoside RT inhibitor; OR, odds ratio; CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.

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1. Introduction

The emergence of drug-resistant human immunodeficiency virus type 1 (HIV-1) among patients under highly active antiretroviral therapy (HAART) limits the successful suppression of HIV-1 replication. Several years after the introduction of HAART, drug-resistant strains are being detected among newly diagnosed HAART-naïve patients, suggesting the transmission of drug-resistant HIV-1 from the treatment-exposed population. Thus, treatment-naïve patients have been recommended by the US Department of Health and Human Services, International AIDS Society-USA, and other drug-resistance testing guidelines to undergo drug resistance testing at diagnosis and before initiation of HAART (DHHS, 2009; Hirsch et al., 2000, 2008). Indeed, choosing effective antiretrovirals according to the results obtained from this testing has led to successful control of HIV-1 infection. Furthermore, the drug resistance testing at diagnosis helps to understand transmission of drug-resistant HIV-1 in HAART-naïve individuals which in turn may help prevent transmission events.

The prevalence of drug-resistant HIV-1 among treatment-naïve patients has been closely monitored and reported from many countries. Before and early in the HAART era, when only mono or dual therapy was available, the prevalence was as high as 10–20% (Boden et al., 1999; Gómez-Cano et al., 1998; Tambussi et al., 1998). However, after the introduction of antiretrovirals with better pharmacokinetics, such as ritonavir-boosted protease inhibitor (PI), the emergence of drug-resistant viruses seemed to decrease (Gallego et al., 2001; Maia Teixeira et al., 2006).

Furthermore, despite the great number of HIV-1-infected patients, the prevalence tended to be low in developing countries where patients had limited or no access to antiretroviral drugs, e.g., 0–4.2% in Africa (Bártolo et al., 2009; Mintsa-Ndong et al., 2009; Ndembi et al., 2008; Pillay et al., 2008), 1.5% in Cambodia (Nouhin et al., 2009), and 2.6% in Vietnam (Ishizaki et al., 2009). In contrast, in countries where antiretroviral drugs are more accessible, the prevalence has been higher, e.g., 5.2% in Thailand (Apisarnthanarak et al., 2008), 9.4% in Taiwan (Chang et al., 2008), 10.0% in India (Lall et al., 2008), 7.8% in Portugal (Palma et al., 2007), 9.0% in Germany (Sagir et al., 2007), 9.5% in Belgium (Vercauteren et al., 2008), 10.9% in France (Chaix et al., 2009), and 15.9% in the US (Eshleman et al., 2007).

In Japan, since the first HIV-1-infected case was identified in 1985, the annual number of reported cases has been increasing every year, reaching 15 451 by the end of 2008. With more people getting infected, larger numbers of patients are starting anti-HIV-1 treatment and the risk of emerging drug-resistant HIV-1 is increasing. To understand the trends in drug-resistant HIV-1 in Japan, a nationwide surveillance project has been in effect since 2003. In our previous report of surveillance results from 2003 to 2004, the prevalence of drug-resistant HIV-1 in newly diagnosed patients was 4.0% (Gatanaga et al., 2007). We have continued collecting and analyzing data from newly diagnosed HIV-1-infected patients at participating clinical and research facilities in Japan. We report here the prevalence of drug-resistant HIV-1 among newly diagnosed therapy-naïve patients between 2003 and 2008.

2. Materials and methods

2.1. Sample

The study population included all the HIV-1-infected patients newly diagnosed between January 2003 and December 2008 at any of the participating HIV/AIDS clinics. Drug resistance genotypic tests were performed at 12 laboratories including 8 clinical laboratories at HIV/AIDS clinics, 3 public health laboratories, and the National Institute of Infectious Diseases. After patients agreed to participate in our surveillance project and gave informed consent, peripheral blood was drawn with EDTA added, and their demographic and clinical information were collected. Demographic information included age, gender, nationality, and risk behavior. Clinical data included HIV-1 viral loads, $CD4^+$ T cell counts, status of hepatitis B and C virus (HBV, HCV) co-infection, baseline sequence data, and drug-resistant amino acid mutations.

This study was conducted according to the principles in the Declaration of Helsinki, and was approved by the ethical committee of the National Institute of Infectious Diseases, Japan. By Japanese law, HIV-1-infected patients must be reported to the Japanese Ministry of Health, Labour, and Welfare upon diagnosis. The numbers reported to the Ministry are considered the "official numbers" of newly diagnosed HIV/AIDS cases, and were used as comparison controls to evaluate our study population.

2.2. Drug resistance genotypic testing

Drug resistance genotypic testing was performed using in-house protocols. Briefly, viral RNA was extracted from patient plasma samples. HIV-1 protease (PR, 1–99 amino acids) and the N-terminal region of reverse transcriptase (RT, 1–240 amino acids) were amplified in reverse transcription polymerase chain reaction (RT-PCR) followed by nested PCR using in-house primer sets. Subsequently, the amplified PCR products were purified and their sequences were analyzed by direct sequencing method using an automated sequencer. The resulting electropherograms were analyzed using commercially available software. The quality of testing methods used at each participating facility was assessed and confirmed for detection of drug-resistant mutations (Fujisaki et al., 2007). Thus, detection of drug-resistant mutations was consistent among facilities.

2.3. Determination of HIV-1 subtypes and drug-resistant HIV-1

HIV-1 subtypes were determined using the sequences of HIV-1 PR and RT genes obtained in the drug resistance genotypic testing explained above. Each sequence was aligned with the reference sequences of HIV-1 subtypes A through K, and circulating recombinant forms (CRFs), all of which were obtained from the Los Alamos HIV Databases (Los Alamos, 2010), using ClustalW, and phylogenetic trees were constructed using the neighbor-joining method with bootstrap value of 1000.

The resulting sequences were compared to that of HXB2 to judge the presence of amino acid mutations. The drug-resistant mutations were determined according to criteria of the HIV Drug Resistance Database of Stanford University (Bennett et al., 2009). Thus, a sample was considered to harbor drug-resistant HIV-1 if it possessed any of the following mutations: in the PR gene, L23I, L24I, D30N, V32I, M46I/L, I47V/A, G48V/M, I50V/L, F53L/Y, I54V/L/M/A/T/S, G73S/T/C/A, L76V, V82A/T/F/S/C/M/L, N83D, I84V/A/C, I85V, N88D/S, and L90M (indicating PI resistance); in the RT gene, M41L, K65R, D67N/G/E, T69D/insertion, K70R/E, L74V/I, V75M/T/A/S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F/I/S/C/D/V/E, K219Q/E/N/R (indicating nucleoside RT inhibitor [NRTI] resistance), and L100I, K101E/P, K103N/S, V106M/A, V179F, Y181C/I/V, Y188L/H/C, G190A/S/E, P225H, M230L (indicating non-nucleoside RT inhibitor [NNRTI] resistance).

2.4. BED assay

The time of HIV-1 seroconversion was estimated in randomly selected samples as recent (within 155 days) or not recent using the BED assay (Calypte HIV-1 BED Incidence EIA, BioRad) according to the Manufacturer's instruction. Briefly, 5 μL of plasma was diluted

with 500 μL of sample diluent in the kit, and the proportion of anti-HIV-1 IgG to a total IgG in the sample was measured by optical density.

2.5. Statistical analysis

Statistical analyses were performed using R software (SAS Institute). Chi-square or Fisher's exact probability tests were used to determine associations among patients' demographic characteristics, nationality, BED assay results, and transmission of drug resistance. The odds ratio (OR) and 95% confidence intervals (CI) were calculated for all the variables. Recent and not-recent seroconversion groups were examined for differences in HIV-1 viral loads by analysis of covariance (ANCOVA), with $CD4^+$ T cell count as the covariate.

3. Results

3.1. Majority of treatment-naïve patients are Japanese men who have sex with men (MSM) in mid-30s

The demographics of the 2573 newly diagnosed HIV-1-infected patients enrolled between 2003 and 2008 are summarized in Table 1. Male (n = 2397, 93.2%), Japanese (90.1%), and those infected through male-to-male sexual contact (68.9%) predominated, and the median age was 35. For the female cases (n = 170), high-risk heterosexual contact was the major risk factor (n = 152, 89.4%), and approximately half were non-Japanese (n = 63, 41.4%). Further analysis showed a significant association between the transmission route and nationality, i.e., most Japanese patients were infected through male-to-male sexual contact, while non-Japanese patients were infected by other routes (OR = 5.60; 95% CI 4.14–7.63; $p < 0.01$) (Table 2). It should be noted that sexual contacts (92.1%) are the major risk factor for HIV-1 infection in Japan. On the other hand, injecting drug usage, one of the high risk factors in other countries, accounts for only 0.4%.

HBV and/or HCV co-infection, an important clinical factor affecting prognosis and treatment of HIV infection (Ockenga et al., 1997; Piroth et al., 2000), was found to have a prevalence of 8.4% of 2101 patients, and 4.7% of 2071, respectively (Table 1). These prevalence rates did not change significantly throughout the study period (supplementary Table 1). HBV co-infection was found to be significantly associated with subtype B (OR = 2.04; $p < 0.05$) or infection through male-to-male sexual contact (OR = 1.66; $p < 0.05$).

3.2. Subtype B HIV-1 predominates in Japan

Of 2573 plasma samples collected during the study period, the sequences of PR and RT genes were successfully amplified and analyzed in 2536 (98.6%) and 2534 (98.5%) samples, respectively. Of these, we examined sequences of the PR-RT region from 2496 cases by phylogenetic tree analysis to determine the distribution of HIV-1 subtypes in Japan. Subtype B HIV-1 was found to predominate among the study population (n = 2194, 87.9%). The remaining non-B subtypes included 210 (8.4%) CRF01_AE, 30 (1.2%) C, 19 (0.8%) CRF02_AG, 18 (0.7%) A, 9 (0.4%) G, 7 (0.3%) F, 5 (0.2%) D, and 1 (0.04%) CRF08_BC (Table 1). In addition, 1 recombinant case of K/C, A/K, and D/B was detected in 2005, 2006, and 2007, respectively. These non-B subtype viruses were found mostly among the heterosexually infected population (223/302, 73.8%). In contrast, subtype B HIV-1 was found in the vast majority of MSM (1700/1773, 95.9%). In terms of nationality, Japanese patients, most of whom were MSM, were infected with subtype B HIV-1. On the other hand, only about a half of non-Japanese patients harbored subtype B HIV-1, and the remaining half were infected with non-B HIV-1, such as CRF01_AE (OR = 8.85; 95% CI 6.46–12.1; $p < 0.01$) (Table 2). This result is reasonable considering that the predominant HIV-1 subtype differs by country, and our study population included many Thais and Malaysians. In addition, this result suggests that subtype B HIV-1 is transmitted in a closed community of MSM, while non-B subtype strains are spread in wider areas among those infected through high-risk heterosexual contacts.

Table 1
Demographic characteristics of newly diagnosed HIV/AIDS patients.

	6-Year total (2573)	
Age		
Average	37.4	
Median	35	
Mode	35	
Quartile (Q1, Q3)	29, 43	
Nationality	*n*	(%)
Japanese	2319	(90.1)
Non-Japanese	225	(8.7)
Asian	83	(3.2)
Oceanian	4	(0.2)
North American	17	(0.7)
South American	58	(2.3)
European	10	(0.4)
African	26	(1.0)
Unspecified[a]	27	(1.0)
Unknown	29	(1.1)
Transmission category		
Male	2397	(93.2)
Male-to-male sexual contact	1773	(68.9)
High-risk heterosexual contact	369	(14.3)
Sexual contact	75	(2.9)
IDU	8	(0.3)
Other[b]	26	(1.0)
Unidentified	146	(5.7)
Female	170	(6.6)
High-risk heterosexual contact	152	(5.9)
IDU	3	(0.1)
Other[b]	5	(0.2)
Unidentified	11	(0.4)
Unknown	6	(0.2)
Unidentified	6	(0.2)
Hepatitis co-infection[c]		
HBV		
(+)	176	(8.4)
(−)	1925	(91.6)
Unknown	472	
HCV		
(+)	98	(4.7)
(−)	1973	(95.3)
Unknown	502	
HIV-1 subtype[c]		
B	2194	(87.9)
non-B	302	(12.1)
AE	210	(8.4)
C	30	(1.2)
AG	19	(0.8)
A	18	(0.7)
G	9	(0.4)
F	7	(0.3)
D	5	(0.2)
Other	4	(0.2)
Unidentified	77	

[a] Unspecified individuals in the nationality category were identified only as of non-Japanese origin.
[b] Other transmission categories include mother-to-child, blood products, transfusion, and needle stick.
[c] Prevalence of subtypes, HBV, and HCV was calculated after omitting the unidentified or unknown data. DU, intravenous drug user; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1.

3.3. Prevalence of drug-resistant HIV-1 is increasing in Japan

A total of 194 cases (7.7%) in the 6-year study period were found to harbor HIV-1 strains with at least one major drug-resistant muta-

Table 2
Characteristics of newly diagnosed Japanese and non-Japanese HIV/AIDS patients.

	Nationality (*n*)			Odds ratio
	Japanese	Non-Japanese	Unknown	
Gender				
Male	2224	151	22	11.45*
Female	95	74	1	
Unknown[b]			6	
Transmission category				
Male-to-male sexual contact	1691	73	9	5.60[a,*]
High-risk heterosexual contact	399	114	7	
Sexual contact	72	4	0	
Other	29	10	2	
Unidentified[b]	128	24	11	
Subtype				
B	2051	118	25	8.85*
Non-B	198	101	3	
Unidentified[b]	70	6	1	
BED assay (*n* = 640)				
Recent	220	13	0	2.31*
Not recent	351	48	8	
Drug-resistant HIV-1				
Detected	173	16	5	1.05
Not detected	2146	209	24	

[a] Odds ratios for the transmission category were calculated between male-to-male sexual contact and other categories which include high-risk heterosexual contact, sexual contact, and other.
[b] Unknown and Unidentified cases were omitted in calculation of odds ratio.
* $p < 0.01$.

tion conferred by PIs, NRTIs, or NNRTIs. The annual prevalence of drug-resistant mutations shown in Fig. 1 had an overall tendency to increase from 5.9% (16/273) in 2003 to 8.3% (50/605) in 2008. The most prevalent mutation in each year was NRTI-associated resistance, with 11 (4.0%), 12 (4.0%), 21 (5.0%), 23 (5.2%), 28 (5.9%), and 23 (3.7%) cases, followed by PI- and NNRTI-associated mutations. PI-resistant major mutations were detected in 63 cases (2.5%), and NNRTI-associated mutations were detected only in 20 cases (0.8%). These data reflect the type of antiretrovirals being prescribed in treated population. In other words, NRTIs have a long history of being prescribed including the period of mono and dual therapy; thus, NRTIs have been more frequently used. As a consequence, NRTI-resistant HIV-1 has emerged and been transmitted more frequently to treatment-naïve patients. Regarding the drug-resistant mutations shown in Table 3, T215 revertants (T215X) (3.2%), M184I/V (0.5%), K103N (0.6%), and M46I/L (1.7%) accounted for the majority of detected mutations in contrast to other muta-

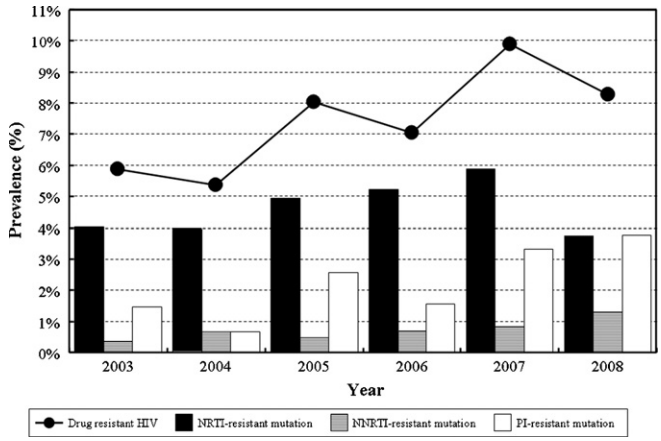

Fig. 1. Annual overall prevalence of drug-resistant HIV-1 (solid circles) in Japan increased in treatment-naïve patients in Japan from 2003 to 2008. The most prevalent mutation in each year was associated with resistance to nucleoside reverse transcriptase inhibitor (NRTI) treatment. Annual prevalence of drug-resistance mutations was categorized by antiretroviral drug class (NRTIs, solid black bars; non-nucleoside reverse transcriptase inhibitors [NNRTIs], horizontally striped bars; protease inhibitors [PIs], solid white bars). Drug-resistant HIV-1 was counted once even when the strain contained multiple drug-resistant mutations. Each drug-resistant mutation was counted even when multiple mutations were detected in one patient.

Table 3
Drug-resistant mutations in newly diagnosed HIV/AIDS patients, by class of antiretroviral drugs.

	6-Year total (2573)	
	n	(%)
NRTI[a]		
M41L	11	(0.4)
K65R	1	(0.0)
D67N/G/E	7	(0.3)
T69D	8	(0.3)
69INS	1	(0.0)
K70R/E	2	(0.1)
L74V/I	3	(0.1)
V75A/M	2	(0.1)
Y115F	3	(0.1)
M184V/I	12	(0.5)
L210W	5	(0.2)
T215X	81	(3.2)
K219Q/E/N/R	4	(0.2)
NNRTI[a]		
L100I	1	(0.0)
K101E	2	(0.1)
K103N	14	(0.6)
V106A/M	1	(0.0)
Y181C/I/V	3	(0.1)
P225H	1	(0.0)
P236L	1	(0.0)
PI[a]		
L24I	1	(0.0)
D30N	5	(0.2)
V32I	3	(0.1)
M46I/L	44	(1.7)
I47V/A	2	(0.1)
V82A/L	2	(0.1)
I85V	5	(0.2)
N88D/S	7	(0.3)
L90M	4	(0.2)

[a] Numbers of cases and the proportions in parentheses are listed.

Table 4
Predictive factors for transmission of drug-resistant HIV-1.

	Drug-resistant HIV-1 (*n*)		Odds ratio
	(+)	(−)	
Gender			
Male	183	2214	1.92
Female	7	163	
Nationality			
Japanese	173	2146	1.05
Non-Japanese	16	209	
Transmission category			
Male-to-male sexual contact	130	1643	0.91
High-risk heterosexual contact	37	484	
Sexual contact	15	60	
Other	1	40	
Unidentified[a]	11	152	
Subtype			
B	180	2014	2.36**
Non-B	11	291	
Unidentified	3	77	

[a] For calculation of odds ratio, unidentified cases were omitted.
** $p < 0.01$.

tions that were detected only sporadically throughout the study period (supplementary Table 2).

Analysis of possible predictive factors for transmission of drug-resistant HIV-1 showed that individuals infected with subtype B HIV-1 had a significantly higher tendency to harbor drug-resistant HIV-1 than non-B subtypes (OR = 2.36; 95% CI = 1.27–4.88; $p < 0.01$) (Table 4). Other possible predictive factors, including male gender (OR = 1.92; 95% CI = 0.89–4.93; $p = 0.1$), Japanese nationality (OR = 1.05; 95% CI = 0.62–1.92; $p = 1$), and MSM behavior (OR = 0.91; 95% CI 0.66–1.26; $p = 0.57$), were not significant predictive factors in our study population. These results indicate that the chance of getting infected with drug-resistant HIV-1 was the same for anyone regardless of gender, nationality, or risk behavior.

3.4. MSM are diagnosed earlier than heterosexually infected individuals

To examine awareness of HIV infection, especially of risk behavior, and to characterize HIV-testing patterns among the HIV-infected population, we estimated the time of seroconversion by quantifying the amount of anti-HIV antibody in plasma samples. Of 640 randomly selected samples in 2007 and 2008, 233 (36.4%) were classified by BED assay with a cut-off value of 0.8 as recently infected (<155-day seroconversion), while the remaining 407 (63.4%) were classified as not recently infected (Table 5). For the recently and not recently infected groups, the average $CD4^+$ T cell count and HIV-1 viral load were 285 and 215 cells/μL and 5.1×10^5 and 1.4×10^5 copies/mL, respectively. Recently infected individuals were shown by ANCOVA with $CD4^+$ T cell counts as the covariate, to have significantly higher HIV-1 viral loads than not recently infected cases (Fig. 2). These data support that the BED assay had precisely determined early infected cases.

With respect to risk behavior, the highest rate of recent infection was in MSM (39.2%), followed by either homo- or heterosexual contacts (38.9%), and heterosexual contacts (25.0%). No patients infected through a risk behavior other than sexual contacts were categorized as recently infected. Whereas 37.8% of male patients were determined to be recently infected, only 13.8% of female patients were categorized as recently infected. These findings were reinforced by statistical analysis. Recent HIV-1 infection was significantly predicted by male gender (OR = 3.79; 95% CI 1.29–15.17; $p < 0.01$), MSM behavior (OR = 1.67; 95% CI = 1.11–2.54; $p = 0.01$), Japanese nationality (OR = 2.31; 95% CI 1.20–4.76; $p < 0.01$), and infection with subtype B HIV-1 (OR = 5.64; 95% CI = 2.37–16.33; $p < 0.01$) (Table 5). In other words, Japanese males, especially those who were MSM, were more aware of being at high risk of HIV-1 infection and got tested more often than non-Japanese. In contrast, females, individuals of non-Japanese origin, heterosexuals, and non-subtype-B-infected persons, had low awareness of the risks of HIV-1 infection.

Table 5
Predictive factors for recent or not-recent seroconversion determined by BED assay, $n = 640$.

	Seroconversion (*n*)		Odds ratio
	Recent ($n = 233$)	Not recent ($n = 407$)	
Gender			
Male	229	377	3.79**
Female	4	25	
Unknown[b]	0	5	
Nationality			
Japanese	220	351	2.31**
Non-Japanese	13	48	
Unknown[b]	0	8	
Transmission category			
Male-to-male sexual contact	189	293	1.67[a,*]
High-risk heterosexual contact	24	70	
Sexual contact	7	11	
Other	0	4	
Unidentified[b]	13	29	
Subtype			
B	224	350	5.64**
Non-B	6	53	
Unidentified[b]	3	4	
Drug-resistant HIV			
Detected	14	37	0.64
Not detected	219	370	

[a] Odds ratio for the transmission category was calculated between male-to-male sexual contact and other categories which include high-risk heterosexual contact, sexual contact, and other.
[b] Unknown or unidentified cases were omitted in calculation of odds ratio.
* $p < 0.05$.
** $p < 0.01$.

Regarding associations between the time of diagnosis and drug-resistant HIV transmission event, time of diagnosis did not differ significantly between those harboring and those not harboring drug-resistant HIV-1 (OR = 0.64; 95% CI = 0.31–1.24; $p = 0.18$) (Table 5), suggesting that transmission of drug-resistant HIV-1 is not a recent trend, but has been ongoing since the first antiretroviral, AZT, was introduced in 1986.

4. Discussion

Our study results show that the proportion of drug-resistant HIV-1 among newly diagnosed cases in Japan increased slightly (by 2.4%) from 2003 to 2008, with fluctuations from year to year. Drug-resistant HIV-1 in HAART-naïve patients are transmitted from HAART-experienced patients with inadequate adherence or from other treatment-naïve individuals with drug-resistant strains, but not yet diagnosed or tested for drug-resistant HIV-1 (de Mendoza et al., 2005). Hence, drug-resistant mutations detected in the naïve population should be tightly related to trends in antiretroviral use in the treated population. Antiretrovirals available in the early days of the HAART era, especially, had short half-lives and low genetic barriers for drug resistance acquisition, making the viruses easily resistance prone. On the other hand, new antiretroviral drugs, such as lopinavir, atazanavir, amprenavir and darunavir, have been developed so that they have improved pharmacokinetics and higher genetic barriers, thus the viruses have less chance of developing drug resistance (Dunn et al., 2008; Lima et al., 2008; Zajdenverg et al., 2009). In the present study, we found that drug-resistant mutations detected among treatment-naïve patients were

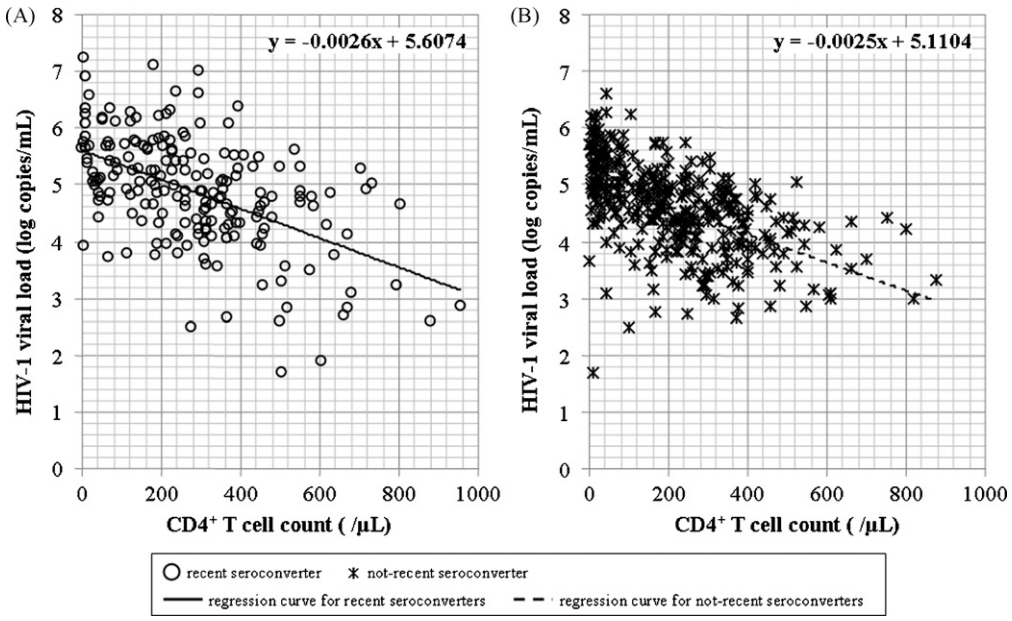

Fig. 2. Scatter plots of viral load and CD4+ T cell counts for (A) recently seroconverted patients (○), and (B) not recently seroconverted patients (*) determined by BED assay. Regression curves and their equations are shown for each group.

associated especially with antiretrovirals used prior to and early in the HAART era. It should be noted that contrary to the reports from the United States and many of European countries (Audelin et al., 2009; Vercauteren et al., 2009; Wheeler et al., 2010), the prevalence of NNRTI-resistant variants have been determined to be low in Japan, less than 1% in the study period 2003–2007 and 1.3% in 2008 being the highest. This difference is due to the situation in Japan that delavirdine had never been used and even nevirapine is only rarely prescribed. Nonetheless, strains with T215X, M46I/L, K103N, and M184V/I mutations were detected every year, suggesting that these strains are stably maintained in individuals and in high-risk populations even under antiretroviral drug-free environments. This finding is supported by the insignificant difference in prevalence of drug-resistant HIV-1 between recently and not recently infected groups. These results raise the concern that such drug-resistant strains may have become some epidemic strains actively transmitted among newly diagnosed HIV/AIDS patients. Furthermore, considering the presence of low frequent variants, the prevalence of drug-resistant mutations in this report may be higher if more sensitive techniques, such as allele-specific PCR and ultra-deep sequencing, are applied to test the samples (Halvas et al., 2010; Varghese et al., 2009). Further studies employing such techniques are needed to understand the detailed epidemic in Japan.

In investigating predictive factors for transmission of drug-resistant strains, we found that the only predictive factor was subtype B HIV-1 (OR = 2.36, $p < 0.01$). The lower transmission risk of drug-resistant strains in non-B HIV-1 can be explained by patients' countries of origin. We observed a significant relationship between non-B subtype HIV-1 and non-Japanese patients, most of whom were from developing countries with limited access to antiretrovirals. Thus, our finding agrees with reports of low prevalence drug-resistant HIV-1 transmission in developing countries (Bártolo et al., 2009; Ishizaki et al., 2009; Mintsa-Ndong et al., 2009; Ndembi et al., 2008; Nouhin et al., 2009; Pillay et al., 2008).

Interestingly, a high proportion of Japanese MSM was diagnosed as recently infected compared to patients of non-Japanese origin, and females determined by BED assay. This result may be due to successful prevention programs targeting the MSM community, so that they have become more aware of their risks of HIV-1 infection. On the other hand, many of non-Japanese patients are seen at hospitals long after HIV infection is established. In addition, women tend to be ignorant of the risks of HIV infection, thus they are often diagnosed upon a prenatal HIV screening test.

Although MSM was not a predictive factor for transmission, this group included 130 cases with drug-resistant HIV-1, the highest prevalence among all the transmission categories. Therefore, those who are involved in prevention programs should take one step further to remind the MSM community about drug-resistant HIV-1 and the limited choice of effective antiretrovirals. HIV-1 transmission has been reported to be prevented in models that assessed the effect of HIV-1 testing for wider populations and immediate initiation of antiretroviral therapy (Granich et al., 2009). Although this model seems very appealing, our results suggest the importance of not forgetting the emergence and transmission of drug-resistant HIV-1 and the limited selection of antiretroviral drugs. It is important to continue surveying newly diagnosed HIV/AIDS patients to keep track of trends in drug-resistant HIV-1 transmission, to reveal high-risk populations with low awareness of HIV infection, to propose effective programs to prevent transmission of drug-resistant HIV-1, and to develop antiretroviral drugs with improved pharmacokinetics/pharmacodynamics. All these efforts may bring us one step closer to eradicating HIV-1.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.antiviral.2010.07.008.

References

Apisarnthanarak, A., Jirayasethpong, T., Sa-nguansilp, C., Thongprapai, H., Kittihanukul, C., Kamudamas, A., Tungsathapornpong, A., Mundy, L.M., 2008. Antiretroviral drug resistance among antiretroviral-naive persons with recent HIV infection in Thailand. HIV Med. 9, 322–325.

Audelin, A.M., Lohse, N., Obel, N., Gerstoft, J., Jørgensen, L.B., 2009. The incidence rate of HIV type-1 drug resistance in patients on antiretroviral therapy: a nationwide population-based Danish cohort study 1999–2005. Antivir. Ther. 14, 995–1000.

Bártolo, I., Rocha, C., Bartolomeu, J., Gama, A., Fonseca, M., Mendes, A., Cristina, F., Thamm, S., Epalanga, M., Silva, P.C., Taveira, N., 2009. Antiretroviral drug resistance surveillance among treatment-naive human immunodeficiency virus type 1-infected individuals in Angola: evidence for low level of transmitted drug resistance. Antimicrob. Agents Chemother. 53, 3156–3158.

Bennett, D.E., Camacho, R.J., Otelea, D., Kuritzkes, D.R., Fleury, H., Kiuchi, M., Heneine, W., Kantor, R., Jordan, M.R., Schapiro, J.M., Vandamme, A.M., Sandstrom, P., Boucher, C.A., van de Vijver, D., Rhee, S.Y., Liu, T.F., Pillay, D., Shafer, R.W., 2009. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One 4, e4724.

Boden, D., Hurley, A., Zhang, L., Cao, Y., Guo, Y., Jones, E., Tsay, J., Ip, J., Farthing, C., Limoli, K., Parkin, N., Markowitz, M., 1999. HIV-1 drug resistance in newly infected individuals. JAMA 282, 1135–1141.

Chaix, M.L., Descamps, D., Wirden, M., Bocket, L., Delaugerre, C., Tamalet, C., Schneider, V., Izopet, J., Masquelier, B., Rouzioux, C., Meyer, L., Costagliola, D., 2009. Stable frequency of HIV-1 transmitted drug resistance in patients at the time of primary infection over 1996–2006 in France. AIDS 23, 717–724.

Chang, S.Y., Chen, M.Y., Lee, C.N., Sun, H.Y., Ko, W., Chang, S.F., Chang, K.L., Hsieh, S.M., Sheng, W.H., Liu, W.C., Wu, C.H., Kao, C.L., Hung, C.C., Chang, S.C., 2008. Trends of antiretroviral drug resistance in treatment-naive patients with human immunodeficiency virus type 1 infection in Taiwan. J. Antimicrob. Chemother. 61, 689–693.

de Mendoza, C., Rodriguez, C., Eiros, J.M., Colomina, J., Garcia, F., Leiva, P., Torre-Cisneros, J., Aguero, J., Pedreira, J., Viciana, I., Corral, A., del Romero, J., Ortiz de Lejarazu, R., Soriano, V., 2005. Antiretroviral recommendations may influence the rate of transmission of drug-resistant HIV type 1. Clin. Infect. Dis. 41, 227–232.

DHHS, 2009. Panel on antiretroviral guidelines for adults and adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents.

Dunn, D., Geretti, A.M., Green, H., Fearnhill, E., Pozniak, A., Churchill, D., Pillay, D., Sabin, C., Phillips, A., 2008. Population trends in the prevalence and patterns of protease resistance related to exposure to unboosted and boosted protease inhibitors. Antivir. Ther. 13, 771–777.

Eshleman, S.H., Husnik, M., Hudelson, S., Donnell, D., Huang, Y., Huang, W., Hart, S., Jackson, B., Coates, T., Chesney, M., Koblin, B., 2007. Antiretroviral drug resistance, HIV-1 tropism, and HIV-1 subtype among men who have sex with men with recent HIV-1 infection. AIDS 21, 1165–1174.

Fujisaki, S., Fujisaki, S., Ibe, S., Asagi, T., Itoh, T., Yoshida, S., Koike, T., Oie, M., Konda, M., Sadamasu, K., Nagashima, M., Gatanaga, H., Matsuda, M., Ueda, M., Masakane, A., Hata, M., Mizogami, Y., Mori, H., Minami, R., Okada, K., Watanabe, K., Shirasaka, T., Oka, S., Sugiura, W., Kaneda, T., 2007. Performance and quality assurance of genotypic drug-resistance testing for human immunodeficiency virus type 1 in Japan. Jpn. J. Infect. Dis. 60, 113–117.

Gallego, O., Ruiz, L., Vallejo, A., Ferrer, E., Rubio, A., Clotet, B., Leal, M., Soriano, V., 2001. Changes in the rate of genotypic resistance to antiretroviral drugs in Spain. AIDS 15, 1894–1896.

Gatanaga, H., Ibe, S., Matsuda, M., Yoshida, S., Asagi, T., Kondo, M., Sadamasu, K., Tsukada, H., Masakane, A., Mori, H., Takata, N., Minami, R., Tateyama, M., Koike, T., Itoh, T., Imai, M., Nagashima, M., Gejyo, F., Ueda, M., Hamaguchi, M., Kojima, Y., Shirasaka, T., Kimura, A., Yamamoto, M., Fujita, J., Oka, S., Sugiura, W., 2007. Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan. Antiviral Res. 75, 75–82.

Gómez-Cano, M., Rubio, A., Puig, T., Pérez-Olmeda, M., Ruiz, L., Soriano, V., Pineda, J.A., Zamora, L., Xaus, N., Clotet, B., Leal, M., 1998. Prevalence of genotypic resistance to nucleoside analogues in antiretroviral-naive and antiretroviral-experienced HIV-infected patients in Spain. AIDS 12, 1015–1020.

Granich, R.M., Gilks, C.F., Dye, C., De Cock, K.M., Williams, B.G., 2009. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. Lancet 373, 48–57.

Halvas, E.K., Wiegand, A., Boltz, V.F., Kearney, M., Nissley, D., Wantman, M., Hammer, S.M., Palmer, S., Vaida, F., Coffin, J.M., Mellors, J.W., 2010. Low frequency nonnucleoside reverse-transcriptase inhibitor-resistant variants contribute to failure of efavirenz-containing regimens in treatment-experienced patients. J. Infect. Dis. 201, 672–680.

Hirsch, M.S., Brun-Vézinet, F., D'Aquila, R.T., Hammer, S.M., Johnson, V.A., Kuritzkes, D.R., Loveday, C., Mellors, J.W., Clotet, B., Conway, B., Demeter, L.M., Vella, S., Jacobsen, D.M., Richman, D.D., 2000. Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society-USA Panel. JAMA 283, 2417–2426.

Hirsch, M.S., Günthard, H.F., Schapiro, J.M., Brun-Vézinet, F., Clotet, B., Hammer, S.M., Johnson, V.A., Kuritzkes, D.R., Mellors, J.W., Pillay, D., Yeni, P.G., Jacobsen, D.M., Richman, D.D., 2008. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. Clin. Infect. Dis. 47, 266–285.

Ishizaki, A., Cuong, N.H., Thuc, P.V., Trung, N.V., Saijoh, K., Kageyama, S., Ishigaki, K., Tanuma, J., Oka, S., Ichimura, H., 2009. Profile of HIV type 1 infection and genotypic resistance mutations to antiretroviral drugs in treatment-naive HIV type 1-infected individuals in Hai Phong, Viet Nam. AIDS Res. Hum. Retroviruses 25, 175–182.

Lall, M., Gupta, R.M., Sen, S., Kapila, K., Tripathy, S.P., Paranjape, R.S., 2008. Profile of primary resistance in HIV-1-infected treatment-naive individuals from Western India. AIDS Res. Hum. Retroviruses 24, 987–990.

Lima, V.D., Gill, V.S., Yip, B., Hogg, R.S., Montaner, J.S., Harrigan, P.R., 2008. Increased resilience to the development of drug resistance with modern boosted protease inhibitor-based highly active antiretroviral therapy. J. Infect. Dis. 198, 51–58.

Los-Alamos, 2010. HIV Databases, http://www.hiv.lanl.gov/content/index.

Maia Teixeira, S.L., Bastos, F.I., Hacker, M.A., Guimarães, M.L., Morgado, M.G., 2006. Trends in drug resistance mutations in antiretroviral-naïve intravenous drug users of Rio de Janeiro. J. Med. Virol. 78, 764–769.

Mintsa-Ndong, A., Caron, M., Plantier, J.C., Makuwa, M., Le Hello, S., Courgnaud, V., Roques, P., Kazanji, M., 2009. High HIV Type 1 prevalence and wide genetic diversity with dominance of recombinant strains but low level of antiretroviral drug-resistance mutations in untreated patients in northeast Gabon, Central Africa. AIDS Res. Hum. Retroviruses 25, 411–418.

Ndembi, N., Lyagoba, F., Nanteza, B., Kushemererwa, G., Serwanga, J., Katongole-Mbidde, E., Grosskurth, H., Kaleebu, P., 2008. Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe Uganda. AIDS Res. Hum. Retroviruses 24, 889–895.

Nouhin, J., Ngin, S., Martin, P.R., Marcy, O., Kruy, L., Ariey, F., Peeters, M., Chaix, M.L., Ayouba, A., Nerrienet, E., 2009. Low prevalence of drug resistance transmitted virus in HIV Type 1-infected ARV-naive patients in Cambodia. AIDS Res. Hum. Retroviruses 25, 543–545.

Ockenga, J., Tillmann, H.L., Trautwein, C., Stoll, M., Manns, M.P., Schmidt, R.E., 1997. Hepatitis B and C in HIV-infected patients. Prevalence and prognostic value. J. Hepatol. 27, 18–24.

Palma, A.C., Araújo, F., Duque, V., Borges, F., Paixão, M.T., Camacho, R., 2007. Molecular epidemiology and prevalence of drug resistance-associated mutations in newly diagnosed HIV-1 patients in Portugal. Infect. Genet. Evol. 7, 391–398.

Pillay, V., Ledwaba, J., Hunt, G., Rakgotho, M., Singh, B., Makubalo, L., Bennett, D.E., Puren, A., Morris, L., 2008. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. Antivir. Ther. 13 (Suppl. (2)), 101–107.

Piroth, L., Grappin, M., Cuzin, L., Mouton, Y., Bouchard, O., Raffi, F., Rey, D., Peyramond, D., Gourdon, F., Drobacheff, C., Lombart, M.L., Lucht, F., Besnier, J.M., Bernard, L., Chavanet, P., Portier, H., 2000. Hepatitis C virus co-infection is a negative prognostic factor for clinical evolution in human immunodeficiency virus-positive patients. J. Viral Hepat. 7, 302–308.

Sagir, A., Oette, M., Kaiser, R., Däumer, M., Fätkenheuer, G., Rockstroh, J.K., Knechten, H., Schmutz, G., Hower, M., Emmelkamp, J., Pfister, H., Haussinger, D., 2007. Trends of prevalence of primary HIV drug resistance in Germany. J. Antimicrob. Chemother. 60, 843–848.

Tambussi, G., Boeri, E., Carrera, P., Gianotti, N., Lazzarin, A., 1998. Prevalence of mutation associated to resistance with nucleoside analogues in a cohort of naïve HIV-1 positive subjects during the period 1984–1997. J. Biol. Regul. Homeost. Agents 12, 32–34.

Varghese, V., Shahriar, R., Rhee, S.Y., Liu, T., Simen, B.B., Egholm, M., Hanczaruk, B., Blake, L.A., Gharizadeh, B., Babrzadeh, F., Bachmann, M.H., Fessel, W.J., Shafer, R.W., 2009. Minority variants associated with transmitted and acquired HIV-1 nonnucleoside reverse transcriptase inhibitor resistance: implications for the use of second-generation nonnucleoside reverse transcriptase inhibitors. J. Acquir. Immune Defic. Syndr. 52, 309–315.

Vercauteren, J., Derdelinckx, I., Sasse, A., Bogaert, M., Ceunen, H., De Roo, A., De Wit, S., Deforche, K., Echahidi, F., Fransen, K., Goffard, J.C., Goubau, P., Goudeseune, E., Yombi, J.C., Lacor, P., Liesnard, C., Moutschen, M., Pierard, D., Rens, R., Schrooten, Y., Vaira, D., Van den Heuvel, A., Van Der Gucht, B., Van Ranst, M., Van Wijngaerden, E., Vandercam, B., Vekemans, M., Verhofstede, C., Clumeck, N., Vandamme, A.M., Van Laethem, K., 2008. Prevalence and epidemiology of HIV type 1 drug resistance among newly diagnosed therapy-naive patients in Belgium from 2003 to 2006. AIDS Res. Hum. Retroviruses 24, 355–362.

Vercauteren, J., Wensing, A.M., van de Vijver, D.A., Albert, J., Balotta, C., Hamouda, O., Kücherer, C., Struck, D., Schmit, J.C., Asjö, B., Bruckova, M., Camacho, R.J., Clotet, B., Coughlan, S., Grossman, Z., Horban, A., Korn, K., Kostrikis, L., Nielsen, C., Paraskevis, D., Poljak, M., Puchhammer-Stockl, E., Riva, C., Ruiz, L., Salminen, M., Schuurman, R., Sonnerborg, A., Stanekova, D., Stanojevic, M., Vandamme,

A.M., Boucher, C.A., 2009. Transmission of drug-resistant HIV-1 is stabilizing in Europe. J. Infect. Dis. 200, 1503–1508.
Wheeler, W.H., Ziebell, R.A., Zabina, H., Pieniazek, D., Prejean, J., Bodnar, U.R., Mahle, K.C., Heneine, W., Johnson, J.A., Hall, H.I., 2010. Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses U.S.-2006. AIDS 24, 1203–1212.
Zajdenverg, R., Badal-Faesen, S., Andrade-Villanueva, J., 2009. Lopinavir/ritonavir (LPV/r) tablets administered once- (QD) or twice-daily (BID) with NRTIs in antiretroviral-experienced HIV-1 infected subjects: results of a 48-week randomized trial (study M06-802). In: 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Cape Town, South Africa.