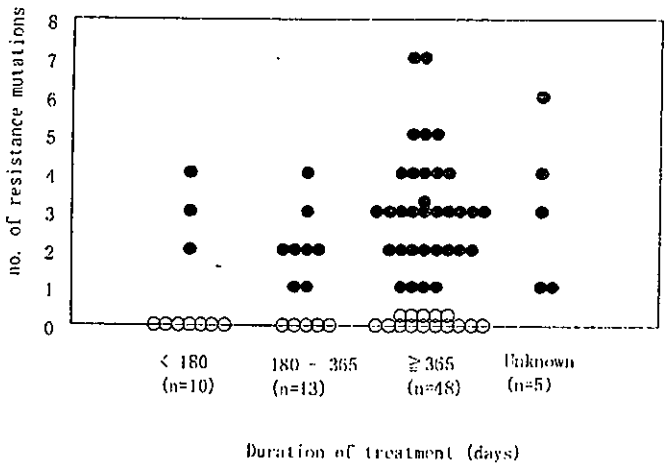

FIGURE 2. Prevalence of AZT, ddI, or ddC resistance mutations in relation to the duration of the dual therapy. Open circles indicate samples without resistance mutations.

Drug Resistance Mutations After Stopping Antiretroviral Drug Therapy

The interval between stopping dual therapy and the time of sampling was also associated with the presence of drug resistance mutations. Twenty-six individuals who had been substantially exposed to dual therapy for >180 days were off therapy at the time of sampling. Seven individuals (27%) were off therapy for >180 days and 19 (73%) were off therapy for <180 days. Drug-resistant viruses were detected in only 1 individual (14%) in the former group but 7 individuals (37%) in the latter group.

Evaluation of M41L and K70R MS-PCR in Detecting AZT-Resistant Strains

We then compared direct sequence methods with M41L and K70R MS-PCR in 96 antiretroviral drug–experienced individuals for whom both sequence and MS-PCR results were available (Table 2). Overall concordant rate for codon 41 was 96.9% (93/96) where M41I was regarded as a mutant type and concordant rate for codon 70 was 92.7% (89/96). Discordant results were seen mainly in the samples that were determined as mutant type by the MS-PCR and as wild type by the sequencing method.

To study the sensitivity of M41L and K70R MS-PCR as a screening strategy in detecting AZT-resistant strains, we defined the AZT-resistant strains as viruses with at least one AZT resistance mutation, which was detected by the sequencing method. Out of the 96 plasma samples that were tested by both the sequencing and the MS-PCR methods, 52 samples had no AZT resistance mutation and 44 samples had at least one AZT resistance mutation and were regarded as containing AZT-resistant viruses. Of the 44 samples with AZT-resistant viruses, the M41L and K70R MS-PCR detected either M41L or K70R mutation in 38 samples, resulting in the sensitivity of the M41L and K70R MS-PCR in detecting the AZT-resistant viruses at 86.4%. The number of AZT resistance mutations related to the detection rate of AZT resistance mutations by the MS-PCR (Table 3). When the viruses had multiple mutations, the sensitivity of the M41L and K70R MS-PCR was considerably higher. Of 39 samples containing HIV-1 with more than one AZT resistance mutation, 37 samples (94.5%) were diagnosed as having resistant viruses by the M41L and K70R MS-PCR.

TABLE 2. Comparison Between MS-PCR and Sequencing Results

Codon 41 Mutations			
	Sequencing Results		
	M	L	I
MS-PCR results			
Wild	84	1*	0
Mutant	2	7	2

*Sequence result of this patient showed a mixed type of M and L; it turned out to be mutant type when MS-PCR experiment was repeated.

Codon 70 Mutations		
	Sequencing Results	
	K	R
MS-PCR results		
Wild	65	1
Mutant	6	24

Screening AZT-Resistant Viruses Among Antiretroviral Drug–Naive Individuals in Northern Thailand

We applied the M41L and K70R MS-PCR to the screening of 292 antiretroviral drug–naive HIV-1–infected individu-

TABLE 3. The Sensitivity of M41L and K70R MS-PCR in Detecting AZT Resistance Mutations

AZT Resistance Mutations, n	Total	M41L and K70R MS-PCR Results		
		Wild	Mutant	Sensitivity
0	52	52	0	—
1	5	4	1	20%
2	14	1	13	92.9%
3	11	1	10	90.9%
≥4	14	0	14	100%

als attending the Lampang Hospital for the existence of AZT drug-resistant viruses. There were 271 individuals (92.8%) who were known to be infected with HIV-1 via the heterosexual route. We found 2 patients (0.7%) who carried mutant viruses: one had M41L and the other had K70R mutation. Later it was noted that these 2 patients, as well as their spouses, had never received any antiretroviral drugs but both had participated in clinical trials of herbal medicine in the past.

DISCUSSION

Our observation showed that AZT, ddI, or ddC resistance mutations were found in >50% of individuals who had received dual therapy. The prevalence of drug-resistant viruses was higher among individuals who had received the drugs for a longer period, as previously reported.[18,19] We attribute the high prevalence of resistant viruses to the fact that the dual therapy was suboptimal. Clinicians working in government hospitals, however, did not have other options because the more efficient antiretroviral therapy such as triple or quadruple therapy was not affordable for most patients when this study was conducted.[7] Recently, access to multiple antiretroviral drugs has been dramatically improved, because the Government Pharmaceutical Organization (GPO) started the production of generic antiretroviral drugs known as "GPOvir," which is a combined tablet of stavudine, lamivudine, and nevirapine. We nevertheless anticipate that individuals who had already had viruses resistant to NRTI dual therapy may not gain as much benefit from the generic medicine as antiretroviral drug–naive individuals do.

The most common mutations observed in this study were D67N, K70R, and T215Y/F, and we found few mutations at codons 65, 74, 108, 151, and 184. Such patterns of NRTI resistance mutations are similar to the patterns in CRF01_AE infection as well as in subtype B infections that have been reported in our previous report.[4] M184V mutation was often found in our previous study but not in the current study. We think that this difference reflects on the rare use of 3TC in Thailand when this study was conducted. Our current study, though a cross-sectional observation, showed several associations among resistance mutations such as D67N and M41L or K70R, T215Y/F and M41L in Thai strains as known in subtype B infection.[20,21]

We found a high concordance rate of MS-PCR with the sequencing method in detecting M41L and K70R point mutations. The finding is compatible with previous papers.[16,17] Discordant results between the MS-PCR and sequencing method were seen in some samples, most of which showed mutant type by the MS-PCR but wild type by the sequencing method. We think that such discordances are due to the greater sensitivity of MS-PCR for detecting a minor virus population than the sequencing method. However, a high sensitivity and specificity of detecting 2 particular point mutations do not specifically justify the application of M41L and K70R MS-PCR for the screening of AZT-resistant viruses in the field. D67N and T215Y/F mutations are very common but it is technically difficult to establish MS-PCR specific for these mutations due to a higher degree of polymorphism around the mutation sites. Our data showed that these mutations were frequently accompanied by M41L and/or K70R as previously reported in subtype B.[22] Furthermore, we evaluated how efficiently the M41L and K70R MS-PCR could detect AZT-resistant viruses that were detected by the sequencing. The overall sensitivity was reasonably high particularly among the viruses with multiple drug resistance mutations.

This is the first report that addressed the transmission of drug-resistant HIV-1 using a large number of samples in Thailand. We found that the prevalence of HIV-1 strains with either M41L or K70R mutation was as low as 0.7% among our drug-naive population. Considering that the overall sensitivity of the MS-PCR for detecting HIV-1 with any AZT resistance mutation was 86.4%, the prevalence of AZT-resistant HIV-1 was estimated to be 0.8%, which is still very low. There is still the concern that the low prevalence of resistant virus could be a consequence of the fact that the resistance to AZT in the drug-naive population was often associated with mutations at codon 60 or 215. To exclude this possibility, we further tested 60 samples, which were randomly selected from the drug-naive samples and confirmed that none had drug resistance mutations at these sites. The majority (127/292) of drug-naive individuals (43.5%) were initially diagnosed as HIV infected in 1997 or before, when the PMTCT program started in the region, and many were likely to have been infected several years prior to their first diagnosis of HIV infection. Thus, our result may not show an effect, which could have been triggered by the PMTCT program. A report from the United Kingdom suggests that transmission of drug-resistant HIV-1 is increasing.[23] We believe that our report is important in providing the baseline information on AZT-resistant HIV-1.

There has not been a consensus on the strategy of monitoring the transmission of drug-resistant HIV-1 in developing countries. Detecting individuals with primary viremia is ideal but not practical. In our study, we surveyed a drug-naive population for the presence of drug-resistant viruses. One concern with this approach is that drug-resistant viruses, which are generally less fit, might have been overwhelmed by the wild-type viruses in the absence of antiretroviral drug pressure because drug-resistant viruses among drug-treated individuals disappear following the interruption of antiretroviral therapy.[24] However, a recently published paper showed 2 cases of transmission of drug-resistant HIV-1 in which the resistant genotypes remained as a dominant population for a prolonged period in the absence of antiretroviral therapy.[25] Another way to monitor the spread of antiretroviral drug–resistant viruses is to screen infected individuals shortly after they receive antiretroviral therapy, which selects a minor population of insidious

resistant viruses, before de novo resistance mutations occur. Further studies are needed.

This study demonstrates that it is feasible to apply MS-PCR techniques for screening a large number of field samples for the presence of AZT-resistant viruses in Thailand. Taking into account the enormous benefits of MS-PCR such as much lower cost, ease of use, no requirement of automated sequencers, and higher sensitivity of detecting a minor virus population, we think that the M41L and K70R MS-PCR is a useful technique for the screening of AZT-resistant HIV-1 in epidemiologic surveys in developing countries. Recently, GPOvir has become widely available in Thailand. As the patterns of drug-resistant mutations against 3TC and nevirapine are relatively simple, we propose that MS-PCR technique should be considered for monitoring viruses resistant to this combination of antiretroviral drugs.

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