CHAPTER V

DISCUSSION

The clinical use of agents such as AZT to treat Human Immunodeficiency Virus (HIV) infection has prompted considerable interest in drug resistance. HIV isolates from individuals during long-term treatment with AZT frequently show reduced susceptibility to the drug, as determined by cell culture assay of virus isolates. The phenotypic changes in AZT-resistant viruses are associated with multiple nucleotide changes which conferring specific amino acid substitution in the reverse transcriptase (RT) of HIV at codon 41, 67, 70, 215, and 219. One mutation commonly associated with isolates of reduced sensitivity is at codon 215 (Thr^{215}--> Tyr or Phe). [17,95]

To investigate the clinical significance of AZT resistance, a rapid and less laborous assay to analyse large-scale HIV-1 infected individuals is required, preferably without the need to isolate HIV by coculturing with peripheral blood mononuclear cells (PBMCs). The proven association between the degree of AZT resistance and the number of specific mutation in RT has provided a rational basis for using genetic assay to assess AZT-sensitivity. [22,24,25] A genotypic analysis of codon 215 mutant by selective RT-PCR has been developed and used for clinical studies. [117,118]

In Thailand, most of the individuals infected with HIV-1 are heterosexuals and the majority carries HIV-1 subtype E. [116] Much has been reported of the HIV-1 AZT resistance in North America and Europe where HIV-1 subtype B predominates. [4,116] A recent study in Spain showed that the incidence of AZT resistance was increased from 10% in 1991-1992 to 30% in 1995-1996.[119] However, little is known about the HIV-1 AZT resistance in the other parts of world, particularly in regards to other subtypes.

The objective of this study is to determine the prevalence of codon 215 mutation genotype in both AZT-naive and AZT-experienced Thai patients with HIV-1 infection using genetic analysis. The approach to detect this point mutation is by the use of a "selective" PCR procedure. As described,[112] oligonucleotide primers were used to enable differential priming of cDNA which was converted by RT, from wild type and mutant genome. These primers are used separately with appropriate paired common primers (AS62/L1M), and a specific PCR product is generated only when the 3' end of a selective primer (WD 215 or MT 215/ANMER B) exactly matches the target sequence. The present study, however showed that the previously described outer primer (L1M) of the selective PCR[112] failed to amplified HIV-1 subtype E, but not subtype B. (Figure 2) It is suggested that in HIV-1 subtype E nucleotide sequence of the RT gene at this region (position 2554-2556 of primer L1M) is varied from that of subtype B. When the "modified" seminested, selective PCR was developed by which AS62/ANMER B was used as the first round PCR, both HIV-1 subtype E and B could be amplified and it made codon 215 genotypic analysis possible in HIV-1 infected Thai patients.

The prevalences of AZT resistant genotype in both AZT-naive and AZT-experienced Thai subjects with HIV-1 infection were summarized in Table IV. The codon 215 mutant was not observed in the group I (AZT-naive), while 11 of the 50 (22%) subjects from the group II (AZT-experienced < 6 months), and 21 of the 50 (42%) subjects from the group III (AZT-experienced > 6 months) carried the codon 215 mutant. The average baseline CD4 cell counts of the patients with the AZT resistant genotype were 63.6 cells/μL in the group II and 173.4 cells/μL in the group III as compared to that of the patients with wild type which were 132.5 and 225.6 cells/μL in the group II and III, respectively ($p < 0.05$, unpaired student t-test) as shown in Figure 5. It demonstrated that the patients who had a much lower of CD4+ T lymphocytes will develop AZT-resistant mutant genotype faster than ones who had a higher CD4+ T lymphocytes. This observation suggests that there is a positive correlation between advanced disease or lower CD4 cell count and the risk of AZT-resistance, as has been reported in patients infected with subtype B. In the prospective

study of the 10 patients who received AZT-monotherapy, all isolates acquired before the initiation of AZT treatment were wild type (Table VI). Seven out of the 10 patients (70%), showed detectable of codon 215 mutant within 12 months after AZT therapy (Table VI).

The evidence of a high prevalence of AZT resistant at codon 215 mutant in AZT treated Thai patients supports the idea of "it is the end of monotherapy era". The Thai national guidelines in the clinical use antiretroviral agents, therefore, need to be reconsidered and to include the "hit hard, hit early" with the combination treatment stategy, to acheive the most effective HIV infection therapy in Thailand.