CHAPTER 4

Discussion

This study determined the prevalence of genotypic drug resistance in newly HIV-1 infected infants in Thailand that participated in NHSO program and received early infant diagnosis (EID) of HIV infection at the Faculty of Associated Medical Sciences (AMS), Chiang Mai University from year 2007 to year 2014.

The most prevalent HIV-1 subtype was CRF01_AE (92.3%), followed by subtype B (7.3%) and C (0.4%). This is similarly to previous studies. CRF01_AE and B subtypes are predominant in Thailand [114]. HIV-1 subtype C were observed in Thai population [115].

Mutations that associated with at least one drug family resistance were detected in 57 (19.9%, 95% CI: 15.4-24.7) of 287 infants. This study revealed quite high level of drug resistance in newly HIV-1 infected infants in Thailand that associated with other reports in the resource-limited countries where the prevalence of resistance to at least one drug family ranged from 4.9 to 45% [107, 108, 116, 117]. The prevalence of DRMs in this study was reported as 5.1%, 95% CI (2.4-7.7) resistant to nucleoside/nucleotide reverse transcriptase inhibitor (NRTI), 9.1%, 95% CI (5.7-12.5) were resistant to non-nucleotide reverse transcriptase inhibitor (NNRTI) and 1.6%, 95% CI (0.3-3.2) demonstrated the resistance to for protease inhibitors (PI).

The genotypes of mutation that related to NRTI drug resistance were K70Q/T/R (1.1%), T215S/N (1.1%), M184V/I (0.7%), V75L (0.7%), M41L (0.4%), D67N (0.4%), T69N (0.4%), L210W (0.4%), and K219E (0.4%) and NNRTI associated resistance genotypes were Y181C/I/CFIS (2.5%), V179D/T (2.2%), K103N (1.4%), E138G/Q (1.4%), A98G (1.1%), G190A (1.1%), V108IV (0.4%), Y188L (0.4%) and F227L (0.4%).

Major protease inhibitor mutations, M46L (1.2%) and V82A (0.4%) and minor PI mutation, K20I (4.1%), L33F (2.9%), T74S (0.8%) and L23I (0.4%) were observed.

K70Q/T/R and M184V/I mutations had been widely reported as major mutation of HIV DRMs among children patients globally [37, 104, 118-120] and Thailand [109-112]. These mutations are responsible for resistance to NRTI drug including AZT, d4T, 3TC and FTC that were frequency used for PMTCT and treatment [12-14, 84], especially AZT and 3TC. Thymidine analogue mutations or TAMs are cross-resistance mutations that confer to the resistance of AZT and d4T drugs. The major TAMs associated with higher levels of resistance are M41L and T215F/Y. The research has identified two common patterns of TAMs (TAM1, TAM2) that tend to cluster together, accounting for high level resistance to AZT and d4T. The TAM1 is more associated with d4T resistance, features M41L, L210W and T215Y. TAM2 features D67N, K70R, T215F, and K219E/Q and is more associated with AZT resistance [121]. In this finding, no evidence of infant who harbored equal or greater than 3 TAMs was found.

The T215S/N that was mostly found in 3 (1.1%) infants is transitional mutation that does not directly reduce NRTI susceptibility but facilitates virus to develop T215Y/F mutations against NRTIs. In addition, T215S have been reported in many finding as transmitted drug resistance mutation in pediatric patients [107, 122, 123].

The high prevalence of NNRTI DRMs with Y181C/I/CFIS, K103N and V179D mutations in the infected infants was also reported in several studies [104, 107, 108, 118-120]. The mutations mostly confer to resistance NVP drugs. The V179D mutation in the infected infant had been discussed [37] as polymorphic mutation that could have arisen spontaneously via the rapid, error-prone mode of HIV replication.

In this research, only major PI DRMs were included in prevalence analysis. Nevertheless, minor PI DRMs were observed in 20 (8.2%) infants. Although, each individual PI mutation tends to have a small impact of virus susceptibility *in vitro*, especially minor mutation, actually every mutation induces a steady decrease in the rate of HIV virological suppression with that PI drug [124]. Moreover, L10V/I polymorphic mutations were observed in 38 infants (15.6%) in the genotypic determination of protease region. The position of mutation on PR region was shown in Figure 4.1. This

higher frequency correlated with the increased frequency of the same polymorphisms that were detected in viruses isolated from Brazilian children, adult patients [125] and also observed in PI-naive HIV-1 CRF01_AE-infected Thai patients [126].

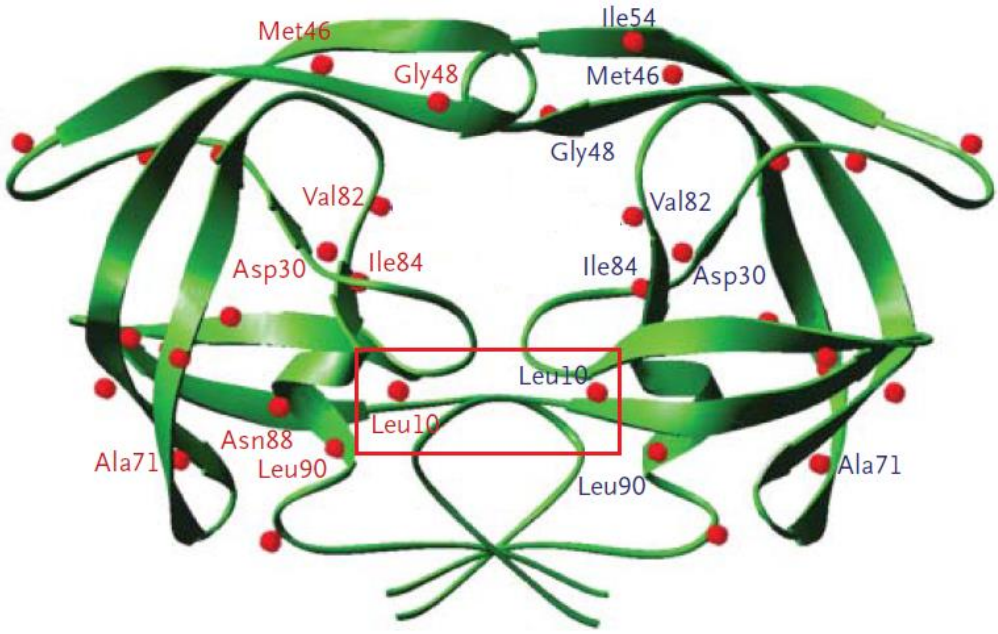

Figure 4.1 The amino acid chains of HIV-1 protease (Modified figure from [18]). The red square cover amino acid position 10 of the protease that mostly occur polymorphic mutation (L10I/V) in this study.

In children, HIV drug resistant can be selected by exposure to ARV drugs used for maternal ART or child prophylaxis in the antenatal, intrapartum and postpartum periods (including during breastfeeding) [127-129]. Furthermore, primary infection with drug resistant virus through MTCT had been documented [36].

Among 287 infants, 238 (82.9%) infants had been exposed to PMTCT. In PMTCT-exposed infants, 4.9% had NRTI mutations, 9.7% had NNRTI mutations and 1.5% had major PI mutations. Among infants who carried NNRTI mutations, 63.6% had exposed sdNVP. The most common mutations were Y181C/I/CIFS and G190A

mutations confer high-level resistance to NVP and confer cross-resistance to EFV in some cases. As previously studied, the used of sdNVP selects resistant viruses at high rates in both mother and infected infants: 10-75% of mother [130,131] and 4-87% of children [132-136]. These resistant viruses can negatively impact the outcome of subsequent NNRTI-based regimen for mothers and the infected infants.

The 27.3% of PMTCT-exposed infants had been exposed to HAART from their mothers. The 4.5%, 10.6% and 3.0% of 66 HAART-exposed infants had NRTI, NNRTI and PI DRMs. The DRMs of seven infants were observed corresponded to ARVs drugs in HAART regimen. The two had no recorded ARV drug type in HAART regimen; however, it is possible that the mother of these infants did receive same type of drug. Because the DRMs that found in these infant accounting for ARV drugs that regularly used for treatment including NVP and EFV.

NRTI DRMs were determined in 4.9% of PMTCT-exposed infants, moreover, only 0.8% had more than one NRTI DRM. Major PI DRMs were observed in 1.5% of PMTCT-exposed infants and no infants had PI DRMs more than one mutations.

In addition, vertical transmission of multidrug-resistant HIV-1 had been described [137]. Because of exposing ARV for long duration in pregnant woman could select multi-variant resistant viruses and transmit to their infants. In this study, three (8%) infants had resistance to NRTI and NNRTI drugs. Two infants had the evidence of ARV exposure which related to occurrence of the DRMs. The initiation of HAART was started at gestational age 154 days in 1 infant and the other infant the initiation time had not available. The remaining one infant had no history of ARV usage.

Besides, forty-five infants had no record of ARV exposure and there were 6 infants carried DRMs. It is possible that may the resistance virus was selected in the mother prior to pregnancy. Furthermore, the mother could be infected with the resistance virus because prevalence of primary HIVDR is approximately 8% among the naïve HIV-infected Thai patients [138]. However, these reasons could not be proved due to the lack of maternal specimens and basic status such as viral load and CD4+ white blood cells count of infants and their mothers. This limitation constrained this research from making any consideration that related to the drug resistance mutation and

risk of perinatal transmission. The others hypothesis were poor storage of records or failure to recall the history of ARV exposures before pregnancies.

Since year 2008 to year 2014, the percentage of PMTCT coverage was continuously increased in the HIV-infected pregnant women and the infants who were born to HIV-infected women: 93.6% to 95.8% of HIV-infected pregnant women and 96.5% to 99.5% of infants who were born to HIV-infected women. [2]. The emergence of DRMs in this study is also continuously raised up. This result indicated that ARV drugs that used for PMTCT had influenced to the emergence of HIV DRMs because HIV resistant variant infection in the infant could transmit from the mother in utero or during intrapartum period and also emerged from exposure of antiretroviral drugs that were given to the infant for PMTCT [139].

Nevertheless, many research demonstrated that drug-resistance selected by sdNVP decayed over time and faded from detection [140-144]. This is similar to the previous studies, the NNRTI DRMs were mostly observed in the infants age less than or equivalent to 3 months. Following Thailand's PMTCT guideline, the infants will receive postnatal ARV prophylaxis around 1 month. In the infant whose age more than 3 months (stopped ARV>1 month), drug resistance variants may displaced by wild-type HIV and may not be detected (below the detection limit) by consensus sequencing [145]. In further study, the next-generation sequencing (NGS) with lower detection limit than consensus sequencing will be perform.

Drug resistance can cause HIV treatment failure in the infected patients. Development of treatment failure in children during ART is frequently found and developed rapidly. This evidence is more associated with extensive drug resistance than treatment failure in adults [146, 147]. Therefore, the correct usage and appropriate formulations of ARV for HIV treatment in children are required.

According to the predicted drug susceptibility in this study, more than 90% of the patients were susceptible to all classes of drugs, while around 10% of patients had the resistance. The NNRTI drugs including NVP and EFV were frequently predicted to be resistant in nearly 6% of patients. For NRTI drugs, 3TC, FTC, AZT and D4T were interpreted as resistance in approximately 2% of patients. The resistance of PI drugs was

shown minimal percentage. The results revealed an absence of predicted resistance to DRV/r (new PI drug generation). NVP, EFV, 3TC, FTC, AZT and D4T were used for children treatment in Thailand [14]. The resistance from this finding had less effect to first-line regimen (AZT(ABC)+3TC+LPV/r) for infected infants (<1-year-old) in Thailand. However, the usage of alternative ART regimen (AZT(d4T)+3TC+NVP) should be considered. Thus, the administration of ARV regimen, regardless of determining of previous ARV exposure by DRMs genotyping assay should be considered in this population, especially NNRTI exposure including sdNVP or NNRTI-based regimen. The resistance testing should be performed in HIV infected infants before ART initiation in order to avoid resistance development and treatment failure.

Although, the resistance testing should be performed in the patients before ART initiation, the cost and logistics that were involved in a HIV DR testing in the infected patients remains challenging in the resource-limited settings. This is the great opportunity to present an in-house genotyping assay to estimate HIV drug resistance. In this study, the in-house genotyping assay was performed to analyze HIV-1 drug resistance in the infants using DBS specimens. The cost of the in-house genotyping assay is approximately 3,300 baht per 1 test that approximately half price of commercial assay such as ViroSeq™ HIV-1 Genotyping System (6,500 Bath per test). Moreover, the turnaround time of this test is approximately 4 days per test. It is less than 50% time consuming of the commercial assay as ViroSeq™ HIV-1 Genotyping System (approximately 7 days).

Now, many studies have reported the successful genotyping of HIV-1 from DBS [148,149]. Moreover, the correlation between genotype data generated using plasma and dried blood spots using in-house methods were determined in few studies [148-151] and demonstrated the high (>78%) genotypic concordance.

Almost 97% of the infant DBS specimens were performed well with in-house genotyping assay but 10 (3%) infant DBS specimens were unable to amplify any RT or PR region in *pol* gene. This situation have been described [152] that the amplification success rates varies widely (53%-92%) and the rates is depending on DBS preparation, storage, and manipulation conditions. In addition, the *pol* is the large fragment of nucleic acid that may particularly sensitive to degradation [152] and the DBS specimens

that used for amplification were stored for long time (average 4 years) prior to performing genotypic test. Furthermore, as it was reported in this finding [151], the patient plasma viral loads was <5,000 RNA copies/ml, the amplification rate was dropped significantly and thus many PCR attempts are required, most likely because of nucleic acid degradation. Unfortunately, the lack of viral load information in this report makes it impossible to describe in this condition. The low viral loads of the infected-infants are likely to be seen in the infants being tested while on postnatal prophylaxis including NVP or AZT [106].

Although, amplifications of proviral DNA sequences from DBS were performed well for determination drug resistance, the genotyping results in some patients with low viral loads (< 400 copies/ml) from DBS specimens may not reflect the current status of replicating viruses circulating in the patient's plasma accurately. However, infected infants <18 months of age usually present with a high viral load because their immune system is immature [106].

Due to the availability of ART that has been greatly expanded in the recent years, the requirement to perform the population-based surveys to assess HIV drug resistance calls for simplified, field-friendly methods for specimen collection, storage, and transport [152]. The DBS sample can be made from blood drawn without special laboratory processing. This study used the remnant DBS samples being tested for early infant diagnosis of HIV infection. Thus, the in-house genotypic drug resistance testing using DBS might be suitable for the test performing in the newly HIV-infected infants in Thailand.