CHAPTER 5

Conclusion and Discussion

We could conclude that HIV-1 subtype CRF01_AE was mainly subtype in Northern Thailand 95.55% follow by subtype B 3.30%, subtype C 0.12% (3 patients), subtype G 0.04% (1 patient), and HIV-1 intersubtype recombination 0.98% (25 patients). Twenty-five HIV-1 intersubtype recombination infected patients composed of 23 HIV-1 recombinant of subtype B and CRF01_AE. We found two patients infected with HIV-1 subtype C/CRF01_AE recombinant and B/C intersubtype recombinant, respectively.

The results illustrated as same as the previous studies that CRF01_AE was circulating in Thailand and URFs and intersubtype recombinants among CRF01_AE, B and C were increasing [10; 148; 149; 152]. The first report in Thailand, during 1994 and 1995 that reported only subtype E 81.8% and B 18.2%, not found inter-subtype recombinant [8]. Until the report of Watanaveeradej in Phase III prime-boost vaccine trial during 1998 to 2001 indicated prevalent HIV-1 infections were mainly CRF01_AE (90%), subtype B (3%), moreover they found 7% either recombinant (two CRF01_AE/B and one CRF01_AE/C recombinant) [148]. The report from the RV144 HIV vaccine trial, presented the subtype distribution: CRF01_AE 91.7%, subtype B 3.5%, B/CRF01_AE recombinants 4.3%, and dual infections 0.5%, moreover they marked CRF01_AE strains were 31% more diverse than those from the 1990s Thai epidemic that informed vaccine immunogen design [149]. Especially in northern Thailand, the study of HIV-1 molecular epidemiology in 2011 suggested that HIV-1 CRF01_AE was predominant (94.8%), followed by subtype B (2.9%) and intersubtype recombination of CRF01_AE with subtype B or subtype C (0.71%). Moreover they reported two patients were migrant and who received HIV-1 subtype C outside Thailand by sexual transmission (0.5%) [152].

Moreover, in this study we reported various pattern of intersubtype recombinants among CRF01_AE, B and C that approximately like CRFs that originated

in Thailand; CRF15_01B, CRF52_01B, and CRF34_01AE. The originated CRFs in Malaysia are CRF33_01AE, CRF48_01B, CRF54_01B, and CRF58_01B. The originated CRFs in Singapore is CRF51_01AE. Moreover HIV-1 B/C intersubtype recombination had the similar recombinant breakpoint to CRF08_BC that originated in China. In HIV-1 subtype C/CRF01_AE recombinant which the result of the phylogenetic analysis showed a mosaic of subtypes C and CRF33_01B, so that this sample would be confirm. HIV-1 subtype C/CRF01_AE recombinant had been detected in Thailand from other studies (Watanaveeradej *et al*., 2006 and Praparattanapan *et al*., 2011). The intersubtype recombination of CRF01_AE with subtype B samples that presented the complex inter-subtype recombination by phylogenetic tree.

The results from *gag-pol*, *env* C2-V3-C3 genotyping and phylogenetic analysis in these intersubtype recombinant, subtype C and G sequences isolates might estimate HIV-1 intersubtype recombination by approximally, so the genetic characterization of HIV-1 subtypes by analyze near-full-genome sequencing can answer completely [156; 157]. Because the new recombinants can be identified by breakpoints that separate different genomic subtype [78; 79] and a study of analyze the position of the breakpoints found that are non-randomly distributed across the genomes of HIV-1 [81]. The borders of the *env* gene around the first exon of *tat*, *vpu* and the beginning of *env*, and the second exon of *tat*, *rev*, and the 3' end of *env* were identified two recombination prone regions or "hot spots". Moreover, comparing the recombination breakpoints across gp120, the C2 region is a hotspot for recombination [82].

Addition we found many HIV-1 intersubtype recombination because HIV maintains a genetic diversity that leads to the diversification of the HIV population. The most of study populations were the treatment-experienced HIV-1 infected patients from Thai citizens and non-Thai citizens who were routinely HIV drug resistances testing and presented plasma viral load $\geq$ 1,000 copies/mL. The genetic variability of HIV-1 generate for evade the host's immunity, and antiretroviral therapy, that depends on high mutation [70] and retroviral recombination rates of the reverse transcriptase enzyme [47], moreover a high replication rate [1]. The previous report said that the wild-type strains are replaced completely by drug resistant virus occurring in plasma within 2-4 weeks [46].

The interested observation was the presence of HIV-1 recombination by *gag-pol* genotype that mean *gag-pol* region had highly mutation that had an effect on HIV diagnosis, prevention, antiretroviral therapy and vaccine development. Analysis of the *pol* gene is used as a genetic marker of initial resistance to antiretroviral drugs that target reverse transcriptase, integrase and protease, so the data set of *pol* sequences is great [77; 158]. Thus, *pol* can be act to track HIV evolution and diversity, even though it is a relatively conserved part of the HIV genome. While genetic diversity of HIV remains a challenge for HIV vaccine development, the rate of diversification can present a problem for efficacy testing [77; 158].

The screening methods to determine HIV-1 subtype and genetic characterization of inter-subtype recombination that were selected for this study were V3 serotyping by subtype-specific enzyme immunoassay (SSEIA). We found inderminate results by SSEIA that composed of the subtype C 2 patients and 7 samples that were subtype CRF01_AE strain by *gag-pol* genotyping, and subtype CRF01_AE, CRF15_AE and the inter-subtype recombinant by phylogenetic analysis. These sample were the treatment-experience patients who presented high level of viral load (>4,500 copies/ml) and CD4 count were range 142-517 cell/mm^3. While naïve-treatment HIV-1 infected patients or newly diagnosed HIV patients were not found inderminate result by SSEIA. Moreover the discoresult results between HIV-1 V3 serotype and *gag-pol* genotype were reported in 241 patients, 73 RNA were genotyped in *env* gp120 C2-V3-C3 region that resulted HIV-1 inter-subtype recombination 7 patients (9.59%) that presented the same subtype between SSEIA and *env* genotyping. While 66 patients (90.37%) were identified subtype follow by *gag-pol* genotype. The reasons of these case were the different principles of the methods, *gag-pol* genotyping identify HIV-1 subtypes by analyze nucleotide sequencing, whereas SSEIA detect specific antibodies based on V3 gp120 region that use the short synthetic peptide corresponding to consensus sequences in the V3 region of HIV-1/2 subtypes. This study used synthetic peptide including subtype B and E (CRF01_AE). These antibodies will be immunized within six months after HIV-1 infection for against to spike envelope. Because of low serum reactivity from the lowering of V3 specific antibodies or recent infection that results SSEIA can not classify subtype. And this antibody can be decreased because the immunological and

virological effects of treatment interruptions in HIV-1 infected patients with ART failure, multidrug-resistant virus or AIDS stage.

This results found the Northern Thai patients who infected with HIV-1 subtype C, G, and intersubtype recombinants of CRF01_AE with subtype B or subtype C that showed hardly control of HIV-1 circulating around Thailand. In recent study, they found two patients were migrant and who received HIV-1 subtype C outside Thailand by sexual transmission [152]. But this study we reported three HIV-1 subtype C infections in both study populations, 1 patient in non-Thai and 2 patients in Thai citizens. Essentially, HIV-1 subtype G infection was found in Thai citizen which is rare subtype in Thailand. While HIV-1 intersubtype recombination of subtype B and CRF01_AE were found in the both study populations, C/CRF01_AE recombinant was found in Thai citizen. And HIV-1 infected patient with B/C recombination was found in non-Thai citizen or immigrant in northern Thailand. When Thailand's participation in AEC and AFTA in 2016 that more travel for trading and traveling, that may effect to the genetic diversity of HIV in Thailand that will increase HIV-1 CRFs that originated from the other country or new CRFs. That the reason to interested study the ability of HIV-1 CRF01_AE and another subtype to recombine in the future [77].

The impact of HIV-1 recombination on diagnosis, antiretroviral treatment, transmission, pathogenesis, clinical management and vaccine development is widely known. Moreover several studies reported that the HIV-1 subtype differences in disease progression, transmission and may relate to viral load [90; 91]. For example, subtypes C and D resulted in more violent diseases, followed by G, CRF01_AE, AG and A [97-100]. Pregnant women infected with subtype C were more likely to transmit HIV to their children than those infected with subtype B [87]. Dual infection might be a result of a higher viral load and a more rapid disease progression [75, 76]. In Chinese HIV infected patients, CRF01_AE was associated with faster disease progression when compared with other subtypes [22]. However, there were the reports showing no differences in disease progression between patients infected with subtypes B and C in Israel [102], patients infected with subtypes A, B, C and D in Sweden [103], and subtypes CRF02_AG and other subtypes in Cameroon [105], additional subtypes B and CRF01_AE in Thailand [104]. The impact of HIV-1 subtype on antiretroviral treatment

in Thailand that CRF01_AE was the mainly subtype, the majority of treated-naive patients did not have drug resistance. Additionally, subtype B strains showed higher number of mutations in both the PR and RT regions than in CRF01 AE. However, the RT gene mutation patterns between B strain and CRF01 AE were basically the same and varied only in T215Y/F and M41L [159].

As we know, HIV-1 recombination plays a role in HIV-1 infections worldwide, about 18-20% of HIV-1 infections around the world [77]. Asia is one of regions that classified as recombination hot-spots. Currently, CRF01_AE that were early recombination, are remaining subtype in south East Asia and Thailand [8]. There are report in West and Central Africa, CRF01_AE was related with many unique and unclassifiable viral sequences [160]. CRF01_AE represents a putative subtype A/E recombinant due to the fact that pure subtype E lineage has never been found [161]. CRF01_AE originated from Central Africa, but is spreading epidemically in Asia [79; 83; 162]. From the previous study, in 2006 CRF01_AE are 84% of all HIV infections in South-East Asia, while Subtype B and other recombinants reported about 4%. In Thailand, Cambodia and Viet Nam, CRF01_AE is spread more than 95% of infections, follow by subtype B and other recombinants. Currently, CRF01_AE are remaining subtype in south East Asia with more prevalent in China and an increasing number of recombinant forms containing CRF01_AE, B, and C subtypes [72; 116]. CRF01_AE dominates in Thailand, Indonesia [147], Laos, Myanmar [72] and Vietnam [163].

There were the previous report based on the Los Alamos HIV Database when had the total 55 CRFs, showed CRF01_AE was circulating 5% of the total HIV-1 infection worldwide. CRF01_AE had also recombined with many other HIV-1 strains and contributes to a total of 9% of all identified CRFs. HIV1 subtype B was predominance worldwide and had the highest recombination frequency (19%) to form novel 55 CRFs. Surprisingly, subtype C, which about 13.8% of HIV-1 infections worldwide, only had recombination frequency (4%) to form novel CRF. While, subtype G which only accounts for 1.1% HIV-1 infection worldwide, had a recombination occurrence of 12% of the total 55 CRFs [77].

The emerging of HIV-1 recombinant forms in many regions around the world, especially ASIA. CRF01_AE may be responsible in shaping the global HIV-1 distribution trend. Pure Subtype did not appear for a long time. New HIV-1 strains have emerged due to either neutral or deleterious recombination of HIV-1 genome from different parental subtypes. Recombination is process of HIV-1 for evolve, emerge and disseminate into world population. It can be imagined that the recombination pattern will continually be driven by the rapid removal of mutations that stop the viral replication [77; 158]. To understand the central role of HIV-1 recombination in the HIV-1 pandemic is essential to provide better insights into the development of next generation HIV vaccines. The current ongoing phylogenetic analysis of HIV sequence variability, which further led to the study of the origin, evolution and spread of HIV-1 recombinants have become more extensive and active.