4037131 SIMI/M : MAJOR: MICROBIOLOGY; M.Sc. (MICROBIOLOGY)
KEY WORDS : PERINATAL HIV TRANSMISSION / DIAGNOSIS / DNA-PCR / QUALITATIVE RNA / *pol* SEQUENCE / ZIDOVUDINE

DAOROONG PIYASATJABOON : PERINATAL TRANSMISSION OF HIV-1 SUBTYPE E : DIAGNOSIS AND NUCLEOTIDE SEQUENCE ANALYSIS OF *pol* GENE. THESIS ADVISORS: RUENGPUNG SUTTHENT, M.D., Ph.D., URAIWAN KOSITA-NONT, Ph.D., KULKUNYA CHOKEPHAIBULKIT, M.D., PRASERT AUEWARAKUL, M.D., Dr. Med. 144 p. ISBN 974-663-587-5

There are approximately 23,000 infants born to HIV-1 infected mothers each year in Thailand. The rate of HIV-1 perinatal transmission in Thailand with no antiretroviral chemoprophylaxis is about 18-24%. Early diagnosis of HIV infection in infants born to HIV-1 infected mothers is critically important for prompt initiation of antiretroviral therapy and other treatments. The sensitivity of HIV coculture and DNA polymerase chain reaction (DNA-PCR) are low in the first few months of life. Because HIV-1 RNA levels rapidly increase after primary infection, the detection of plasma HIV RNA using the qualitative nucleic acid sequence-based amplification (NASBA) may offer a rapid and sensitive alternative method for early diagnosis of perinatal HIV-1 subtype E infection in infants.

To compare the efficacy of qualitative RNA (NASBA) and in house DNA-PCR assays for early diagnosis HIV-1 infection in infants born to HIV-infected mothers, EDTA blood from sixty-three infants, 12 HIV-1 infected infants and 51 HIV-1 uninfected infants, born to HIV-1 infected mothers attending at Siriraj Hospital during December 1997 to December 1998 were collected at the ages of 1-2 months and 4-6 months. The qualitative HIV RNA (NASBA) was more sensitive than DNA-PCR for early diagnosis HIV-1 infection in infants at the first 2 months of life [12 of 12 (100%) vs. 6 of 12 (50%)], respectively; p = 0.031). The specificity of both assays were 100% at the age of 1-2 months. The sensitivity and the specificity of both assays were 100% at the age of 4-6 months.

The amount of HIV-1 subtype E proviral DNA in PBMCs of these 12 HIV-1 infected infants at the age of 4-6 months by using competitive PCR ranged from 140 to 407 copies per $1.0x10^6$ cells, whereas those of 12 HIV-1 infected mothers ranged from 133 to 460 copies per $1.0x10^6$ cells. Symptomatic development of infected infants at age 4-6 months correlated with detection of DNA-PCR at age 1-2 months and proviral DNA quantitation at age 4-6 months. A higher proviral DNA amount was found in infected infants who developed symptoms at age 6 months. This was more than those who did not develop symptoms (p = 0.014).

All isolates from these 12 HIV-1 infected infants at the age of 4-6 months had non-syncytium inducing (NSI) HIV-1 phenotypes, 7 of 12 isolates were macrophage-tropic and 5 of 12 isolates retained both mmacrophage-tropic and T-cell tropic. Seven infants (58%) (C3, C10, Z11, Z24, Z44, Z48, and Z51) developed symptoms and switched from clinical staging EN to A or B within the 6 months of life.

The mean intersample nucleotide distances of the RT region of HIV-1 *pol* gene among 12 HIV-1 subtype E isolates from infected infants and their mothers were 3.16% and 2.81%, respectively. The mean intersample nucleotide distances of the HIV-1 RT region isolates from 7 ZDV treated mothers and their infants (3.07% and 3.34%, respectively) were higher distances than those of 5 non ZDV treated mothers and their infants (2.43% and 2.92%, respectively).

The substitutions of nucleotide sequence in the *pol* (RT) region of HIV-1 subtype E viruses in this study were found to be synonymous substitutions (ds) more than nonsynonymous substitutions (dn), resulting in a very high average ds/dn ratio above 1.

There were no mutations commonly associated with nucleoside analogs RT resistance (M41L, D67N, T69D, K70R, L74V, Y181C, M184V, L210W, T215Y or F, and K219Q) found in *pol* (RT) gene nucleotide sequences of HIV-1 isolates from 12 HIV-1 infected mother-infant pairs, whose samples were collected at 2-6 months after delivery and 14 HIV-1 infected mothers who received short- course ZDV, whose samples were collected at 1-2 days after delivery.

This study provides an early diagnosis lab method for HIV-1 infection in infants born to HIV-1 infected mothers, baseline molecular epidemiological data of RT coding region of *pol* gene for HIV-1 subtype E perinatal transmission in Thailand, and preliminary data about ZDV resistant mutations that were not found in infected mothers who received short-course zidovudine regimen.