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THAWAT SANOHSOMNIENG : GENOTYPIC CHARACTERIZATION OF HIV-1 ISOLATED FROM SEMEN AND BLOOD OF EARLY SEROCONVERT IDUs.

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HIV-1 transmission rates among intravenous drug users (IDUs) in Thailand have remained high since 1988, followed by stable seroprevalence at approximately 30-40 % with continued high incidence. Estimated incidence from IDUs during 1995-1998 was 6.3 per 100 person-years at risk. Beginning in 1988, HIV-1 infections among IDUs consisted mainly of subtype B' virus, but recently the predominant cause of infection by subtype E virus. This study was conducted to determine the quantity, quality, and the relationship of proviral DNA and RNA genome in semen and blood of 46 early seroconvert IDUs. The shedding of HIV-1 DNA was detected by nested PCR. The quantity of proviral DNA and RNA was detected by competitive PCR and NUCLISENS QT TEST (Organon), respectively. Direct sequencing of *gag* and *env* gene was performed by automated DNA sequencing system.

HIV-1 proviral DNA was detected in 100 % of PBMC and 78.3 % of seminal cells by nested PCR *gag* gene. HIV-1 proviral *env* DNA were found 52.8 % of seminal cells samples. HIV-1 RNA was detected in 100 % of seminal fluid. V3 nucleotide sequences were 21.7 % of the IDUs samples were classified as subtype B', and 78.3 % classified as subtype E. There was no correlation between CD4+ cell numbers and HIV-1 RNA level in both plasma and seminal fluid.

Mean of proviral DNA level in PBMC and semen were 75.82 (± 86.29) (range 5.86-321.24) copies/ 10^5 PBMC and 17.81 (± 25.19) (range 2.11-89.13) copies/ 10^5 mononuclear cell, respectively. There was no correlation between HIV-1 proviral DNA level in PBMC and seminal cells, but the concentration of HIV-1 proviral DNA was significantly higher in blood than semen. The concentration of HIV-1 DNA subtype E was no higher than subtype B in blood and semen. The relationship between HIV-1 proviral DNA level of both PBMC and seminal cells was significantly correlated with HIV-1 RNA quantity in plasma and seminal fluid.

The range of HIV-1 RNA level of seminal fluid from 2.48 to 5.27 log copies/ml and mean (\pm SD) was 3.92 (± 4.44) log copies/ml. Mean of HIV-1 RNA subtype B was 3.40 (± 3.46) log copies/ml and subtype E was 4.02 (± 4.49) log copies/ml. HIV-1 RNA level subtype E was not higher than subtype B in semen.

Mean of *gag* gene in intrasubtype E divergence (interperson) and B' divergence was 4.06 % and 2.72 %, respectively. Mean of V3 region subtype E divergence (interperson) and B' divergence was 8.29 % and 16.25 % (± 4.7 %, n=5), respectively. The phylogenetic tree of *gag* gene showed a tight cluster in the blood and semen from the same person. The phylogenetic tree of *env* gene showed a tight cluster within each subtype in semen. The most V3 crown motif predominant in subtype E was GPGQ, and next GPGR, but did not differ in subtype B. The compartmentalization of HIV-1 V3 and *gag* nucleotide divergence between the blood and semen showed a mean of (\pm SD) 3.34% ($\pm 3.85\%$), (0-6.67%, n=5), 0.88% ($\pm 0.54\%$), (0-2.24%, n=14). There is a compartment of HIV-1 in semen and blood (by V3 nucleotide divergence). The V3 protein sequences were not divergent between direct sequencing and cloning. This study might be one explanation in molecular epidemiology of HIV-1 for the need to develop an HIV-1 vaccine in Thailand