4038414 SIMM/D: MAJOR: MICROBIOLOGY; Ph.D. (MEDICAL MICROBIOLOGY)
KEY WORDS : HIV-1 SUBTYPE E, HETEROSEXUAL TRANSMISSION, GENETIC DIVERSITY, BIOTYPE

KWONCHIT SUMRANSURP: GENOTYPIC AND BIOTYPIC CHARACTERIZATION OF HIV-1 SUBTYPE E ISOLATED FROM SEMEN AND CERVICO-VAGINAL FLUID. THESIS ADVISORS: RUENGPUNG SUTTHENT, Ph.D., M.D., CHANTAPONG WASI, M.D., PILAIPAN PUTHAVATANA, Ph. D., PRASERT AUEWARAKUL, M.D., Dr. med., KLEOPHANT THAKERNGPOL, M.Sc. 137 p.
ISBN 974-663-645-6

The present study investigated viral factors that influenced heterosexual transmission of HIV-1 subtype E in 30 males and 30 females. All subjects were asymptomatic at the time of study. EDTA blood, semen, and cervico-vaginal fluid (CVF) were collected. HIV-1 genome detected by PCR amplification of *gag* gene in semen and CVF were found 43.33% and 56.67%, respectively. The HIV-1 isolation by PBMCs coculture method in semen and CVF were found 20.00% and 16.67%, respectively. HIV-1 subtype determination was carried out by heteroduplex mobility assay and found that all of HIV-1 isolates were subtype E. Two blood isolates (M46P and F36P) and 2 genital fluid isolates were tested for infectivity in mucosal cell lines (HeLa, ME180, HT29 and SW837), T-cell lines (SupT1 and MT2) and primary macrophage. HIV-1 blood isolates and genital fluid isolates were not different in mucosal infection. HIV-1 isolated from CVF (F36V) could infect macrophage more efficiently than the virus isolated from the blood of the same individual (F36P).

The ability of HIV-1 subtype E to infect mucosal cell lines was investigated by electron microscopic technique. There were virus attachment and membrane fusion between virus particles and the HeLa cell line, suggesting that HIV-1 subtype E could enter the mucosal cell.

Neutralizing activity in plasma from HIV-1 infected patient (M46 and F36) against viruses from blood (M46P and F36P) and virus from semen (M46S) could be detected. No neutralizing activity of cell free-genital fluids was found.

From the *env* V1-V4 sequences of 16 blood isolates (M06P, M13P, M24P, M30P, M31P, M37P, M42P, M44P, M46P, SP01P, SP03P, F06P, F27P, F36P, F44P, and W18P) and 8 genital fluid isolates (M31S, M46S, F36V, V02V, V08V, V20V, W18V, and W32V), no difference in genetic divergence of these groups was found (12.14±1.64% and 11.73±2.88%, t test p=0.369). Because of differences in genetic divergence of each variable region of *env* gp120, V1/V2, V3, and V4 were analyzed separately. There were differences of pairwise distances of *env* V3 of blood isolates and genital fluid isolates (18.80±6.81% and 14.80±6.92%, t test p=0.006) and nonsynonymous substitution (18.21±2.33 and 13.84±2.40 p<0.0001). There was deglycosylation of V3 protein of blood isolates but glycosylation was conserved in genital fluid isolates. The *env* V3 of blood isolates of females (F06P, F27P, F36P, F44P, and W18P) were more divergent than those of males (M06P, M13P, M24P, M30P, M31P, M37P, M42P, M44P, M46P, SP01P, SP03P), (23.33±8.32% and 17.00±6.06%, Mann-Whitney test, p=0.038). The *env* V3 of blood isolates of females were more divergent than those of CVF isolates (F36V, V02V, V08V, V20V, W18V and W32V), (23.33±8.32% and 14.40±7.55%, Mann-Whitney test, p=0.014).

The intraperson variation of HIV-1 subtype E in blood and genital fluid of each individual were compared and *env* V1-V4 was divergent about 4-6%, suggesting virus compartmentalization of HIV-1 in blood and genital fluids

Intrasample variation of *env* V1-V5 was confirmed by heteroduplex mobility assay. DNA distance of blood isolates (M46P, M31P, F36P, and V16P) and genital fluid isolates (M46S, M31S, F36V and V16V) were 7.80±2.50% and 4.60+3.10%, respectively (Mann-Whitney test p= 0.068). C2-V3 DNA distance of blood isolates (M06P, M31P, M33P, M37P, M41P, M42P, M43P, SP03P, F06P, F33P, F36P, F44P, F46P, V15P, V16P, W18P, and W32P) were higher than those of genital fluid isolates (M31S, M46S, H16S, F36V, V02V, V08V, V20V, W18V, and W32V), (5.90±2.70 and 1.90± 2.40, Mann-Whitney test p=0.002).

In conclusion, the HIV-1 subtype E shedding in genital fluids detected by gag PCR for semen and CVF were 43.33% and 56.67%, and by isolation were 20.00% and 16.67%, respectively. There was no difference in infectivity of HIV-1 from both compartments in mucosal cell lines. Both T- tropic and M-tropic viruses could be found in genital fluids but only T-tropic virus in blood. There was virus compartmentalization in blood and genital fluids due to having a low degree of heterogeneity and a high degree of *env* sequence clustering of HIV-1 genital fluid isolates, while the HIV-1 genital fluid isolates had a high degree of high heterogeneity and a low degree of *env* sequence clustering. The characteristic of HIV-1 subtype E in genital fluids that affected heterosexual transmission was not found.