MATERNAL AND VIRAL FACTORS IN VERTICAL TRANSMISSION OF HIV-1 SUBTYPE E

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Abstract. Vertical transmission of HIV-1 is caused by multifactorial factors. To access the relationship of viral factors involving in perinatal transmission of HIV-1 subtype E, which is the predominant type in Thailand, plasma viral load, blood CD4+ lymphocyte level, heteroduplex mobility, and V3 sequence of the HIV-1 envelope gene were studied in 32 transmitting and 25 non-transmitting mothers. We found that HIV-1 subtype E vertical transmission was strongly associated with high maternal plasma viral RNA ($> 4 \times 10^4$ copies/ml) and high genetic diversity of envelope gene determined by heteroduplex mobility (< 0.9). The variation of nucleotide sequences in envelope gene of subtype E vertical transmission could not determine in V3 region. Hence, plasma viral load and heteroduplex mobility can be used as prediction factors in vertical transmission of HIV-1 subtype E.

INTRODUCTION

It has been predicted that there will be 40 million HIV infected cases worldwide by the year 2000, while over 14 million women are expected to have been infected. The rate of HIV-1 vertical transmission varies from 13-43% globally (Quinn 1996; Peckham and Gibb, 1995). HIV-1 subtype E is found predominantly in Thailand and other Southeast Asian countries and is spread by heterosexual route (Burke *et al*, 1996). Two percent of Thai women enrolled at antenatal clinics are HIV seropositive and the perinatal transmission rate is 24% with subtype E predominant (Shaffer *et al*, 1996; Chotpitayasnindh *et al*, 1996). There are approximately 1 million livebirths per year in Thailand, an estimated 20,000 HIV-1 seropositive women give birth and 4,800 are HIV infected children every year.

The factors influencing transfer of virus from the mother to newborn are not well understood. The transmission was reported to be correlated with high viral load, genetic features and virulence of viruses, a reduced CD4+ cell count, and low levels or absence of antibodies in the mother to the viral envelope (especially V3 region) (Newell and Peckham, 1993; Thea Donald *et al*, 1996; Rossi *et al*, 1989; Burgarde *et al*, 1992; St Louis *et al*, 1993; Fenyo *et al*, 1989; Scarlatti *et al*, 1993 a, b, c). Also, mutations in the V3 region could potentially affect mother-to-child transmission, since the V3 loop is an important determinant for virus neutralization and cellular tropism (Javaherian *et al*, 1990; Levy, 1994).

An important limitation of the previous studies is the reliance upon HIV subtype B. In the case of sub-type E which rapid spreads in Thailand by heterosexual transmission and vertical transmission from mothers to children, there is a need for further confirmation whether those factors may affect the risk of vertical transmission.

MATERIALS AND METHODS

Subjects

EDTA blood specimens were collected from 57 HIV-1 seropositive mothers and their children, who were attending at the pediatric clinic, Siriraj Hospital during 1993-1995. The mothers were divided into two groups, 32 transmitting (T) and 25 non-transmitting (NT) mothers after knowing the infection status of infants at 6 months old by PCR method. Blood samples were taken from the moth-

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ers at the initial visit, which varied from 1 day to 30 days after birth and from babies every 4-6 months for 2 years. The infants were diagnosed as HIV-1 infection by PCR positivity and seropositivity at age 6 months and at age 18 months, respectively.

DNA preparation

Viral DNA for PCR amplification was isolated from peripheral blood mononuclear cells (PBMCs) of the patients. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation and plasma was kept at -80°C for viral load assay. PBMCs were lysed in PCR-lysis buffer (10mM Tris-HCl pH 8.3, 2.5mM $MgCl_2$, 50 mM KCl, 0.45% NP-40, 0.45% Tween 20 and 100 µg/ml proteinase K) at a concentration of 10^7 cell per ml of lysis buffer for 1 hour at 56°C followed by 10 minutes at 95°C in water bath to inactivate the enzyme.

PCR amplification

HIV DNA was amplified from the lysate by nested PCR as described in the procedure of Delwart *et al* (1993). The outer primers were ED3 (5'-TTAGGCATCTCCTATGGCAGGAAGAAGCGG at position 5956-5985 of the HXB2CG genome, (Genbank accession number K03455) and ED14 (5'-TCTTGCCTGGCGCTGTTTGATGCCCCA-GAC, position 7960-7931). The inner primers were ED5 (5'-ATGGGATCAAAGCCTAAAGCCA-TGTG, position 6556-6581) and ED12 (5-AGTGCTTCCTGCTGCTCCCAAGAACCC-AAG, position 7822-7792). Nested PCRs were carried out with a total volume of 50 µl, containing 10 µl of the cell lysate, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.25 mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer and 2.5 units of Taq DNA polymerase (Gibco-BRL, USA). The amplifications were carried out in a Perkin-Elmer Thermocycler for 30 cycles with step of 94°C for 15 seconds, 55°C for 45 seconds, 72°C for 1 minute and final extension at 72°C for 5 minutes and 2 µl of the first reaction product were used as template in a second round PCR with the inner primers under the same conditions.

Heteroduplex mobility assay (HMA)

5 µl of nested PCR product with primers ED3/ED14 and ED5/ED12 was used to form heteroduplexes with either 5 µl of those from reference strains of HIV-1 subtype B and E, to identify HIV-1 subtype, or with 5 µl of water, to identify intra-quasispecies. 1.1 µl of heteroduplex annealing buffer (1X = 0.1 M NaCl, 10 mM Tris-HCl pH 7.8, and 2 mM EDTA) was added to the reaction which was denatured at 94°C for 2 minutes and cooled rapidly by transferring to ice with water. The heteroduplex reaction mixture was mixed with 3 µl of 5X Ficoll/loading dye and loaded on to a 5% non-denaturing polyacrylamide gel. The electro-phoresis was performed at a constant 200 V for 6 hours. The gel was photographed under UV light after stain with ethidium bromide.

Heteroduplex mobility was calculated as the distance of migration of the heteroduplex bands divided by the distance of migration of the homoduplex bands. DNA distance (%) can be calculated from heteroduplex data as the following relationship:

DNA distance = -ln[(mobility-0.106)/0.91]/7.86.

Cloning and sequencing

The second-round PCR reactions were detected for the DNA amplification product (approximately 1,200 bp) by horizontal 0.8% agarose gel electrophoresis in TBE buffer at 100 V for 30 minutes. The PCR products were purified from gel slices with Geneclean (Bio 101 Inc, La Jolla, California) and cloned into PCR II vector using the TA Cloning system (Invitrogen, San Diego, California) according to the procedure in the manufacturer's instructions. Clones with the inserted *env* amplified DNA size were selected and sequenced by dideoxy-sequencing with Sequenase Version 2.2 (United States Biochemical Corp, Cleveland, Ohio). The sequencing primer used for V3 was 5'-CTGTT-AAATGGCAGTCTAGCT (sequence derived from HIV-1 LAI, Genbank accession no K02013; primer CB 207) (Ou *et al*, 1993).

Sequence analysis

The 105 nucleotide sequences of the V3 region of the HIV-1 *env* gene from 17 mother-infant pairs were translated into the corresponding amino acid sequences and aligned by using DNASIS version 2.1 (Hitachi Software Engineering, Japan). Pairwise