The Detection of HIV Antigen and Clinical Manifestations in HIV Infected Children

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INTRODUCTION
At the present time the diagnosis of HIV infection depends on detection of anti-HIV-1 antibody by either enzyme linked immunosorbent assay (ELISA), gelatin particle agglutination, or other methods. It is difficult to diagnose the perinatal transmission of human immunodeficiency virus in infants by these methods, because all infants born to seropositive mothers have detectable HIV-IgG antibodies which may persist for as long as 15 months(1,2). The HIV infection in infants could be diagnosed by endogenous antibody synthesis(3,4), HIV-IgA antibodies(5), HIV isolation from peripheral blood culture and molecular detection of HIV nucleic acid by polymerase chain reaction(6-8), but these techniques are relatively complicated to perform routinely. The technique to detect HIV-p24 by ELISA technique is at present a conventional technique(9-11). Some investigators suggested that HIV-p24 antigen in infants born from infected mothers can be correlated with the stages of disease(7,12). The objective of this study was to study the correlation of HIV-p24 antigen with clinical stages in infants born from seropositive mothers at pediatric ward, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University.

MATERIALS AND METHODS
1. Clinical samples were obtained from infants in pediatric ward, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University who have anti-HIV-IgG positive by ELISA technique (Vironostika, Organon, Holland) and confirmatory test by Western Blot (Novapath, Biorad, U.S.A.). These samples were collected during January 1991 to January 1992. All subjects were classified into 3 groups by clinical symptoms (10,13); group 1 (class PO: indeterminate), group 2 (class P2 : symptomatic) included symptomatic HIV-infected patients with disease not mentioned in AIDS surveillance definition, group 3 (class P2: symptomatic) included patients with AIDS according to the surveillance definition for acquired immunodeficiency syndrome(14).

2. Anti-HIV 1 detection: The enzyme linked immunosorbent assay (Vironostika, Organon, Holland) was performed according to the direction of the manufacturer. Diluted sample was added into each well. Following an incubation at 37°C for 30 minutes the strip was washed four times with washing buffer. Color was developed for 30 minutes after adding substrate and the reaction was stopped by adding 2 N H₂SO₄, the optical density was measured by using filter at 492 nm. Cut off value for positive and negative result was determined under the manufacturer instruction.

3. Anti-HIV 1 confirmatory: The Western Blot technique was performed according to the direction of the manufacturer (Novapath, Biorad, U.S.A.) for each sample that was positive for anti-HIV antibody by ELISA. The sample was considered to be positive by Western Blot technique if that sample showed antibodies against envelope band at least 1 band and core protein at least 1 band.

4. HIV-p24 antigen detection: The HIVAG-1-Monoclonal EIA (Abbott, U.S.A.) polystyrene beads coated with monoclonal antibody to HIV-1 p24 were incubated with serum samples. After incubation in 40°C waterbath for 3 hours unbound solution were aspirated and the beads were washed by washing buffer. Rabbit anti HIV-p24 antibody was added and incubated 40°C for 1 hour. After aspiration and washing, goat anti-rabbit antibody conjugated with horseradish peroxidase was added and incubated at 40°C for 1 hour. Unbound enzyme conjugate was aspirated and the beads were washed by washing buffer, substrate solution was added to the beads. After incubation, a yellow orange color was developed in proportion to the amount of HIV-1 p24 antigen. The reaction was stopped by addition of 1 N sulfuric acid and intensity of color was measured using a spectrophotometer. The sample showed the absorbance value equal to or greater than calculated cut off value was considered to be reactive for HIV-p24 antigen. All repeated reactive specimens were considered to be positive for HIV-p24 antigen.

RESULTS
There were 31 blood samples from 31 children, all of their mothers were asymptotically HIV infected cases. The children were all under 1 year old (averaged age 3.03 ± 2.92 months) and could be classified as follows: eight babies into group 1 (class PO), 10 cases in group 2 (class P2 with diseases not mentioned in AIDS definition) and 13 cases in group 3 (class P2 with AIDS definition). The ten cases in group 2 could be further classified into subclass A, normal immune function in 4 cases; subclass D, secondary infectious diseases in 6 cases; subclass E, primary infectious diseases in 2 cases; subclass F, infant with AIDS in 3 cases.
Table 1 Numbers of HIV p24 antigen positive patients in each groups.

<table>
<thead>
<tr>
<th>Group (Classification*)</th>
<th>Number of patients</th>
<th>HIV-p24 antigen positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (P0 : indeterminate)</td>
<td>8</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>2 (P2 : symptomatic)</td>
<td>10</td>
<td>4 (40.0)</td>
</tr>
<tr>
<td>3 (P2 : AIDS)</td>
<td>13</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>10 (32.2)</td>
</tr>
</tbody>
</table>

*Centers for Disease Control. Classification system for human immunodeficiency virus infection in children under 13 years of age. MMWR 1987; 36:225-30.

discussion

From this study, 31 blood samples from HIV-positive children were HIV p24 antigen positive in 10 cases (32.2%) which was similar to previous studies of Palomba E, et al and Paul DA, et al, whose studies found the prevalence of HIV antigen positivity in children born from infected mothers 33.3 and 34.8 per cent respectively(1,10). When we focused on the correlation of the detection of HIV antigen and clinical manifestations, we found HIV antigen in class PO 25.0%, class P2 with diseases not mentioned in AIDS surveillance definition 40.0 per cent and class P2 with AIDS 30.8 per cent as compared to previous studies which showed the prevalence of HIV antigen to be 0, 16.6, 36.3 per cent and 19.0, 42.0, 69.0 per cent respectively(1,10). The prevalence of HIV antigen in AIDS group was not different from the study of Palomba but the prevalence in class PO and class P2 (subclass A and subclass D) in this study was higher. We found no significant correlation between the HIV p24 antigen detection and clinical manifestations. The limitation of this study was the classification of the studied children could not be done definitely due to lack of pathologic studies. Some patients presented with severe bacterial infections and rapidly progressed to death and were classified in symptomatic HIV infected group but not AIDS, although these group of patients met the WHO criterias for diagnosis of AIDS(15). The discordant of classification system may result in the falsely high prevalence of HIV p24 antigen detection in group 2.

Further study using the method which pretreat serum with low pH acid for immune complex dissociation for increasing the sensitivity or using purified fluorescein isothiocyanate conjugated antibody as well as other sophisticated technique i.e. PCR, viral culture, HIV IgA antibody may help in diagnosis of HIV infection in children under 15 months of age.

REFERENCES


