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Clinical course of children of human immunodeficiency virus-infected mothers in Taiwan

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The objective of this study was to describe the clinical courses of infants born to human immunodeficiency virus (HIV)-infected mothers in Taiwan. Eleven children, including 1 set of twins, born to 10 HIV-infected mothers were included in the study. HIV antibodies were assayed and HIV-1 polymerase chain reaction (HIV-PCR) or virus cultures were performed; HIV infection was established when there were at least 2 separate positive results of HIV-PCR or culture. Three sets of primers detecting LTR-*gag*, *pol* and *env* were used. The viral load of HIV RNA was measured and used as an indicator of the treatment response. Two of the children were HIV-infected and received combination therapy, including 2 kinds of nucleoside analogue reverse transcriptase inhibitors plus 1 protease inhibitor. Neither of these children exhibited HIV-related symptoms or signs during the study period. Both mothers of the infected children were Taiwanese and their HIV infection status was not known during pregnancy. In contrast, HIV infection was found in early pregnancy in the 4 women living in Taiwan who were from other countries, all of whom received prophylactic therapy. The other 4 mothers who did not transmit the infection to the infant were Taiwanese, 3 of whom were known to be HIV-seropositive during pregnancy. Based on these results, the vertical rate of transmission was 18% (2/11). Early detection of HIV-infected pregnancy is vital to reduce the incidence of HIV-infected births.

Key words: HIV, polymerase chain reaction, vertical disease transmission

In the past, pediatric infections with the human immunodeficiency virus (HIV) were mainly attributable to virus-contaminated blood transfusions [1]. HIV screening of blood components made this mode of transmission unlikely. Nevertheless, the number of HIV-infected children in the world continues to increase, predominantly through vertical transmission from the mother. Previous studies have demonstrated that vertical transmission can occur in utero, around the time of delivery, or postpartum through breast-feeding [2,3]. Although the exact proportion or efficiency of transmission during these periods is unclear, most evidence suggests that HIV transmission occurs predominantly in utero or perinatally [3]. The transmission rate of HIV from mother to infant when the mother does not receive antiretroviral therapy during pregnancy has been reported to range from 15-20% in the US and Europe to 30-40% in several African countries [4,5]. The transmission rate could be dramatically reduced to less than 10% with antiretroviral therapy beginning from the second trimester of pregancy to labor, elective cesarean section, zidovudine (AZT) prophylaxis given to the baby for 6 weeks, and by avoiding breast-feeding of the baby [6,7].

In Taiwan, no report has been published regarding children born to HIV-infected mothers. In view of the increasing number of HIV carriers in Taiwan, more and more maternal-fetal transmission of HIV-1 infection can be anticipated. Sooner or later, physicians in Taiwan will encounter the problem of pediatric HIV-1 infection. We report the clinical courses of 11 children born to HIV-infected mothers, who were followed in our clinic.

Materials and Methods

Sample collection and preparation of plasma and cells

The vacutainer cell preparation tube (CPT) [Becton-Dickinson, Franklin Lakes, NJ, USA] was used to collect 8 mL of whole blood which was then placed in a centrifuge with swinging bucket rotor for 30 minutes

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at 1700 × g (Heraeus Minifuge T). The layer of mononuclear cells was collected with a pipette and transferred to a tube containing 10 mL RPMI 1640 medium (Life Technologies, MD, USA) and centrifuged for 10 mn at 400 × g. The supernatant was removed and 10 mL RPMI 1640 medium and trypan blue were added and the contents mixed. The suspension was transferred onto a KOVA slide (Hycor Biomedical, CA, USA) and the concentration amplified to 10^6 cells/mL.

Viral RNA extraction and cDNA synthesis

300 µL TRIZOL LS Reagent (Life Technologies) was added to 100 mL of the sample and incubated for 5 minutes at room temperature to permit the dissociation of nucleo-protein complexes. 80 µL choloform per 300 µL of TRIZOL LS Reagent was added, followed by incubation at room temperature for 2 to 15 minutes. The samples were then centrifuged at 14,000 rpm for 15 minutes. The aqueous phase was transferred to a clean tube and mixed with isopropyl alcohol and glycogen as carrier. The mixture was then centrifuged again at 14000 rpm for 10 minutes, the RNA pellet washed with 75% ethanol and the sample mixed by vortexing. The RNA pellet was dried briefly and the RNA dissolved in a 20 µL RNase-free water by vortexing and incubating for 10 minutes at 55-60°C. Sufficient cDNA master mix was prepared for cDNA synthesis in the number of samples plus one. The master mix contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 1 mM dNTPs (2 mM dUTP), 1 U/µL RNase inhibitor, 2.5 uM random hexamers and 2.5 U/µL MuLV-RT (Perkin Elmer). The mixtures were incubated for 10 minutes at room temperature to allow the reverse transcriptase to extend the hexamers, for 30 minutes at 42°C to perform the cDNA synthesis, and the reaction was then stopped by incubating the mixtures for 5 minutes at 70°C.

DNA extraction

QIAGEN (QIAmp Blood Kit) protease stock solution and buffer AL (cell lysis buffer) were added to lymphocytes in phosphate-buffered saline and the samples were mixed by vortexing for 15 seconds and incubated at 70°C for 10 minutes. 96-100% ethanol was added to the samples, which were then mixed again by vortexing and centrifuged at 14,000 rpm for 1 minute. Buffer AW (column wash buffer) was added and the DNA eluted by distilled water preheated to 70°C. The DNA was then incubated at room temperature for 1 minute and centrifuged at 14,000 rpm for 1 minute. The DNA was denatured by heating for 15 minutes at 95°C. Amplifications were performed in a GeneAmp polymerase chain reaction (PCR) System 9600 (Perkin Elmer). Details of the PCR primers and the protocols are given in Tables 1 and 2 [8].

Quality control

The performance of the HIV-1 PCR (HIV-PCR) was evaluated during every run using positive and negative

Table 1. Polymerase chain reaction primers for the detection of the human immunodeficiency virus (HIV)-1 proviral or viral genome

Primers	Amplified gene fragment	Sequence (5'-3')
AV10/AV13 (Outer)	LTR-*gag*	TGTGACTCTGGTAACTAGAGATCC CTCAGA/CTGCGAATCGTTCTAGCTCCCTGCTTGCCC
AV11/AV12 (Inner)	LTR-*gag*	TCTAGCAGTGGCGCC/GACGCTCTCGCACCC
H_1POL4235/4238 (Outer)	*pol*	CCCTACAATCCCCAAAGTCAAGG/TACTGCCCCTTCACCTTTCCA
H_1POL4237/4481 (Inner)	*pol*	TAAGACAGCAGTACAAATGGCAG/GCTGTCCCTGTAATAAACCCG
AV18/AV21 (Outer)	*env*	GCACCCACCAAGGCAAAGAGAAGAGTGGTG/TTCCACAGCCAGGACTCTTGCCTGGAGCTG
AV19/AV20 (Inner)	*env*	AGGAAGCACTATGGGC/GCTGCTTGATGCCCCA

Table 2. Polymerase chain reaction (PCR) primers, amplified gene fragments and PCR protocol

Primers	Amplified gene fragment	$MgCl_2$ concentration (mM)	Cycling conditions	Cycle number and final step
AV10-13 (inner)	LTR-*gag*	2	30 seconds, 94ºC 15 seconds, 45ºC 15 seconds, 72ºC	25 cycles + 10 minutes, 72ºC
H_1POL4235-4538 (outer)	*pol*	2	30 seconds, 94ºC 15 seconds, 50ºC 30 seconds, 72ºC	35 cycles + 10 minutes, 72ºC
AV18-21 (outer)	*env*	3	30 seconds, 94ºC 30 seconds, 65ºC	35 cycles + 10 minutes, 72ºC

controls. The negative control contained water added to the PCR mix instead of proviral DNA or cDNA. The positive control contained DNA from ACH2 cells in a background of 10^5 HUT-78 cells. During a correctly performed PCR run, the detection limit should be 5 copies or less. The positive controls of the PCR on cDNA were derived from infectious virus quantified by a commercial assay. During a correctly performed PCR run, 500 copies or less should be detectable.

Subjects

From 1994 to 2001, 11 children born to HIV-1 infected mothers were followed up at the clinics of the Division of Infectious Disease of the Department of Pediatrics at National Taiwan University Hospital (NTUH). Six of these children were born in NTUH. Their mothers were referred to NTUH for obstetrical care soon after detection of their HIV-seropositive status during pregnancy. The other 5 children were born in other hospitals and visited the clinics from the age of 5 to 18 months.

All children were seen at the clinics every 1-2 months on a routine basis, or more frequently if deemed necessary. At each visit, a thorough physical examination was performed to find signs of HIV infection. Other laboratory examinations included complete blood and differential counts, CD4-positive and CD8-positive T cell numbers and ratios, and tests for HIV-1 antibody. HIV-PCR and virus cultures were used to establish the diagnosis of HIV-infection in order to avoid interference from persistent maternal antibodies in the infant's blood [9].

Diagnostic PCR was carried out on proviral HIV-1 DNA using a standardized algorithm based on 3 HIV-1 primer sets. The 3 sets of PCR primers amplify a fragment in the LTR-*gag* gene, *pol* gene and *env* gene. They are situated within conserved regions of the HIV-1 genome and were used for the PCR detection of HIV-1 nucleic acids [8]. A definite positive result was defined as detection in the correct bands on more than 2 PCRs. When no PCR yielded a positive finding, the result was classified as "negative". Otherwise, the nucleic acid detection was defined as indeterminate and rechecked again. When the results of the HIV-PCR were positive, the quantitative viral load of HIV (i.e., the levels of HIV RNA) was measured.

Results

At the time of analysis, the youngest subject was 1 year old and the oldest was 7.5 years. There were 5 boys and 6 girls. The demographic features of these 11 children are listed in Table 3.

All 6 children who had been born in NTUH, including 2 boys and 4 girls, were born via cesarean section (Table 3). One of the 11 children in the series was a preterm baby (gestational age, 32 weeks; body weight, 1564 g) and 1 was small for gestational age. After birth, all of the children received AZT prophylaxis for 6 weeks; medication was stopped if the HIV-PCR results were negative. Mothers were advised not to breast-feed their children. Two mothers of children born in NTUH were Taiwanese and their husbands were also HIV carriers. Of the 4 other mothers, 1 was from China, 1 from Thailand, and 2 were from Vietnam. The husband of the mother from China was the only HIV carrier

Table 3. Clinical features of the infant, paternal HIV status, maternal nationality, and prophylaxis in children born to mothers with HIV infection

Case no.	Gender	Delivery type	Birth condition	Age	HIV status of father	Nationality of mother	AZT prophylaxis
1[a]	Male	C/S	AGA	7 years, 6 months	+	Taiwan	Yes
2[a]	Female	C/S	AGA	3 years, 7 months	+	China	Yes
3[a]	Male	C/S	AGA	1 year, 6 months	-	Vietnam	Yes
4[a]	Female	C/S	AGA	2 years, 6 months	-	Thailand	Yes
5[a]	Female	C/S	PRE/AGA	1 year, 1 month	+	Taiwan	Yes
6[a]	Female	C/S	SGA	1 year	-	Vietnam	Yes
7	Male	NA	NA	4 months	-	China	No
8	Male	C/S	NA	6 months	+	Taiwan	No
9	Female	C/S	NA	6 months	+	Taiwan	No
10	Female	NSD	NA	1 year, 6 months	+	Taiwan	No
11	Male	C/S	NA	5 months	NA	Taiwan	No

Abbreviations: HIV = human immunodeficiency virus; AZT = zidovudine; C/S = cesarean section; AGA = appropriate for gestational age; SGA = small for gestational age; PRE = preterm; NA = not available; NSD = normal spontaneous delivery

[a]Born in National Taiwan University Hospital.

Table 4. Serologic and virologic results in 11 children born to HIV-infected mothers

Case no.	HIV antibody[a]	HIV-PCR	Virus culture
1	+ → - (7 months)	ND	-
2	+ → - (9 months)	ND	-
3	+ → - (15 months)	-	ND
4	+ → - (14 months)	-	ND
5	+ → - (10 months)	-	ND
6	+ → - (8 months)	-	ND
7	+ → - (12 months)	-	ND
8	+	+	ND
9	+ → - (12 months)	-	ND
10	+	+	ND
11	+ → - (6 months)	-	ND

Abbreviations: HIV = human immunodeficiency virus; PCR = polymerase chain reaction; ND = not done
[a]Time of antibody disappearance after birth.

husband. All 6 mothers of the children born in NTUH received antiretroviral therapy after their HIV status was ascertained.

Five children born at hospitals other than NTUH, 3 boys and 2 girls, including 1 pair of heterozygous twins, were referred to NTUH due to the maternal HIV-1 positivity (Table 3). One of these children was born through the vaginal route, 3 via cesarean section, the remaining 1 had an unreported birth mode. These babies were surveyed for HIV at NTUH at the ages of 4, 5, 6 and 18 months, respectively. Three of the mothers were Taiwanese and 1 was from China. Two husbands were also HIV carriers.

Two of the 11 children (cases 8 and 10) were later confirmed to be infected with HIV-1, resulting in a rate of vertical transmission of 2/11 (18%). The other 9 children seroconverted. The serologic and virologic results for these 11 children are shown in Table 4. Maternal antibodies disappeared between the age of 6 and 15 months. None of the 6 children born at NTUH were infected based on the criteria of virus culture and HIV-PCR assay. In contrast, both cases of HIV infection were children born at hospitals other than NTUH. The immunologic and virologic results of the 2 HIV-infected children are listed in Table 5.

Case 8, a male first twin, was confirmed to be HIV-infected at 7 months of age with a high viral load (5×10^6 copies per mL) detected initially. After treatment with combinations of 2 nucleoside analogue reverse transcriptase inhibitors (NRTIs) — AZT and lamivudine (3TC) — plus 1 protease inhibitor, ritonavir, the viral load decreased to an undetectable level after 2 months.

A rebound of the viral load to 5.7×10^4 copies per mL was found 2 months later, probably due to rejection of ritonavir, and the non-NRTI, nevirapine, was prescribed in place of ritonavir. The viral load decreased to undetectable level after changing the medication.

One female born by vaginal delivery (case 10), was confirmed to be HIV-infected at age 18 months, with an initial viral load of 3×10^3 copies per mL. There was also a reversal of the ratio of CD4+/CD8+ T cells. After treatment with 3-agent combination therapy consisting of 2 NRTIs (AZT + 3TC) and 1 protease inhibitor (ritonavir), the viral load fell to undetectable levels and persisted at this low level during follow-up .

Neither of the HIV-infected children developed any HIV-associated symptoms or clinical manifestations such as lymphadenopathy, hepatosplenomegaly, dermatitis, chronic parotid swelling, chronic or persistent

Table 5. Immunologic and virologic results of the 2 HIV-infected children

	Viral load (age, years)	CD4+ count[a]	CD4/CD8+ ratio	Antiretroviral therapy
Case 8	5×10^6 (0.6)	1280	1.11	AZT + 3TC + ritonavir
	444 (0.8)	1970	0.94	
	5.7×10^4 (1)	2990	1.25	AZT + 3TC+ nevirapine
	<400 (1.3)	1870	1.05	AZT + 3TC + indinavir
	500 (1.8)	1660	1.29	
	<400 (2.3)	1200	1.15	
	<50 (2.8)	1210	0.73	
Case 10	3.3×10^3 (1.5)	1630	0.32	AZT + 3TC + ritonavir
	<400 (1.9)	2200	1.32	
	<400 (2.2)	2490	1.41	
	<50 (2.5)	1263	1.43	AZT + 3TC + efavirenz
	<50 (2.9)	1063	1.59	

Abbreviations: HIV = human immunodeficiency virus; AZT = zidovudine; 3TC = lamivudine
[a]CD4+ cells/mL.

diarrhea, chronic or recurrent oral thrush, failure to thrive, or recurrent invasive bacterial infection. No gross abnormality was noted in these 2 children, and growth and neurologic development were within normal limits during follow-up.

Discussion

HIV-infected infants are usually asymptomatic during the first few months of life. The mean age of symptom onset is estimated to be 1 year for perinatally infected infants, but the majority of children remain asymptomatic for more than 5 years [3]. Conventional serologic tests for the diagnosis of HIV infection in infants are inadequate due to the prolonged persistence of maternally-derived transplacental antibodies [9]. Evidence suggests that about half of HIV-infected infants can be identified during the neonatal period using the PCR method. This diagnostic rate increases to 90% by 3 months, and almost 100% by 6 months [8,10,11]. The results of this study support the value of HIV-PCR in the establishment of rapid and correct diagnosis.

Mothers of the 6 children in this series found to be HIV-seropositive during pregnancy were then referred to the obstetrics department of NTUH. All received prophylaxis treatment including maternal treatment with antiretroviral drugs, neonatal prophylaxis with AZT, elective cesarean section, and exclusive formula-feeding for the infants after birth. None of the 6 children born at NTUH were HIV-infected — hence, the above treatment methods appear to have been beneficial.

In contrast, the mothers of the 5 children from hospitals in Taiwan other than NTUH were not found to be HIV carriers during pregnancy and did not receive prophylaxis. Unfortunately, 2 of the 5 children born to these mothers were infected with HIV-1.

One first-born male twin (case 8) was infected, despite lack of infection in his younger sister (case 9). According to previous investigations, the first born of a pair of twins has twice the risk of infection of the second twin [12,13]. The higher infection rates of first twins was linked to prolonged contact with the maternal secretions in the birth canal. However, as the twins in the series were delivered by cesarean section, no vaginal passage was involved. Therefore, it is likely that case 8 was actually infected in utero by a mechanism which remains unclear.

Though the case number in this series was small, the results suggest that prophylactic intervention can reduce the incidence of vertical transmission. It is important for practitioners to detect HIV infection in pregnant women to reduce the possibility of HIV-1 vertical transmission.

It is interesting to note that 4 of the 5 husbands of HIV-infected women from other countries were uninfected, indicating that these mothers became infected by other routes. In Taiwan, marriage to women from Asian and Southeast Asian countries including Vietnam, Thailand, China and Indonesia has been increasing in recent years. Routine and accurate HIV screening before marriage may help identify and reduce the risk of transmission by pregnant women from such areas.

Notably, 4 of the 5 husbands of the Taiwanese women in this series were also HIV carriers. Some mothers do not suspect themselves of being infected with HIV-1 until the development of AIDS in their husbands is disclosed. Hence, the husbands appeared to be the source of infection in most of these cases. Most of the Taiwanese mothers did not receive screening for HIV infection and were thus likely to miss the chance for prophylactic treatment during pregnancy. Universal HIV screening during pregnancy may be considered in the future, especially when the trend of HIV infection is increasing in Taiwan and worldwide.

Both HIV-1 infected children in this series thrived under antiretroviral therapy, including the patient who was likely infected in utero (case 8). No AIDS-defining illness was noted in these patients during follow-up duration and their CD4 counts remained high and their viral loads undetectable.

In summary, in this study of maternal-fetal transmission of HIV-1 infection in Taiwan, the transmission rate was comparable to previous reports in western countries. Antiretroviral therapy should benefit infected Taiwanese children. Screening of pregnant women coming to Taiwan from Southeast Asian countries and China is needed, as well as for the wives of merchants frequenting these areas.

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