HIV-1 SEROPOSITIVE WOMEN IN THE PHILIPPINES: PREGNANCY OUTCOME AND PERINATAL TRANSMISSION OF HIV-1

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Abstract. Annual surveillance studies were initiated in 1985 to determine the incidence and prevalence of HIV-1 infection in female prostitutes registered through the Social Hygiene Clinic System of the Philippine Department of Health. All of the confirmed HIV-1 seropositive women detected in the above surveys who could be contacted were followed up about every three months to monitor their clinical and immunological status. Since we regularly interviewed and examined these HIV-1 seropositive women, we were able to note the occurrence of pregnancies following HIV diagnosis.

By September 1990, 54 HIV-1 seropositive women (aged 18-35) detected from the above surveys had been interviewed and examined. Twenty-six of these HIV-1 positive women had a total of 37 pregnancies. Eight were pregnant at the time of HIV diagnosis: three term deliveries, one premature delivery (PD) at eight months, three abortions, and one lost to follow-up while still pregnant. Five of these eight had repeat pregnancies: two term deliveries, two abortions, and one lost to follow-up while pregnant. Eighteen other women became pregnant one or more times after HIV diagnosis: seven term deliveries, 13 abortions, two PDs, one ectopic pregnancy terminated, one currently pregnant, and one lost to follow-up during pregnancy. There was no clear indication from clinical examinations and CD4+ cell counts that pregnancy exacerbated the course of HIV-1 related disease in these women.

One of the 12 term infants has died and eight have developed non-specific findings that are suggestive but not diagnostic of HIV infection. Most of the 11 babies that are still living were HIV-1 antibody positive by Western blot during their neonatal period. Three infants followed for $\geq$ 20 months reverted from seropositive to seronegative, and have remained seronegative. The Western blot banding profiles for two infants has remained indeterminate after $\geq$ 24 months of follow-up, and the infection status of four other infants is still classified as unknown because they have only been monitored for $\leq$ 12 months. Two infants, however, appear to be truly infected with HIV-1. One of these infants was seronegative at 12 months, but became strongly seropositive by both ELISA and Western blot assay at 16 months. The other infant has been confirmed positive by isolation of virus by peripheral blood lymphocyte co-cultivation at 17 months of age. Our data clearly show the potential for perinatal HIV transmission to occur in this group of women. Further follow up will be required to define the full extent of this problem.

MATERIALS AND METHODS

Background

As of September 1990, the National AIDS Prevention and Control Program had reported 191 HIV infection/AIDS cases in the Philippines (National AIDS Prevention and Control Program, 1990). Among the 160 Filipino cases, 107 (66.9%) were women, most of whom are young and sexually active. Unfortunately, infected females can transmit HIV-1 heterosexually to their partners, and also can pass the virus to their children by the perinatal route as has been well documented in the United

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States and several other countries (McDonald *et al,* 1991). In fact a dramatic rise in the number of pediatric AIDS cases is occurring in the United States in parallel with the increasing number of AIDS cases being reported in women. Over 80% of these pediatric cases were infected by perinatal HIV-1 transmission. We are reporting in this paper on the occurrence of perinatal transmission of HIV-1 in the Philippines.

In 1985, our laboratory initiated annual surveillance studies to determine the incidence and prevalence of HIV infection in female prostitutes registered through the Social Hygiene Clinic System of the Philippine Department of Health (Hayes *et al,* 1990). The HIV study was attached to the syphilis screening programs already in operation. Sera collected from these women were tested for HIV antibody by enzyme immunoassay (EIA) kits (Abbott Laboratories). Sera that were repeatedly reactive in the EIA assay were confirmed positive for HIV antibody a Western blot (WB) assay (Diagnostic Biotechnology) [PTe] Limited). Specimens were classified positive by WB assay when p24 and either gp41 or gp 120/160 bands were present.

All the confirmed HIV antibody positive prostitutes detected in the above surveys were asked to participate in a prospective study to classify and monitor the clinical and immunologic status of their infection. Those who volunteered were instructed to return at 3-month intervals for evaluation. At each visit, the HIV positive women were interviewed by trained personnel using a standard questionnaire to obtain sexual bahavior and pregnancy information. They also were counseled by trained medical personnel to avoid pregnancy because of the high risk of HIV transmission to the child. Since we regularly interviewed and examined these HIV seropositive women, we were able to document the occurrence of pregnancy following HIV diagnosis.

Clinical immunologic evaluation of the HIV-1 positive women and their infants

The clinical status of the women was assessed according to the system of classifying HIV-1 infections established by the Centers for Disease Control (CDC) (1987). This system may be summarized as follows: Group I - signs and symptoms accompanying acute infection; Group II - seropositivity but no signs or symptoms; Group III - persistent polyadenopathy without other findings and Group IV - AIDS and other conditions related to HIV-1 infection. Shortly after birth, each infant's height, weight, and head circumference were recorded as well as the presence of any morphologic abnormalities or clinical signs, such as adenopathy, hepatomegaly, splenomegaly or neurologic problems. The HIV-1 positive women and their infants were examined every three to four months to update the clinical findings and to monitor immunological status.

For virological and serological studies, clotted and heparin-treated blood samples were collected. The sera were screened for HIV-1 antibody by EIA and repeatedly reactive samples were confirmed by the WB assay, as before or by a competitive EIA (ENVACOR) that uses recombinant antigens for the separate detection of anti-p24 and anti-gp41 antibody (Abbott Laboratories). Sera were considered positive when reactivity to both recombinant proteins was present. When sample volumes were adequate, the blood was tested for P24 antigen by a solid-phase capture immunoassay (Abbott Laboratories, North Chicago), and virus isolation was attempted from peripheral blood lymphocytes (PBLs) by co-cultivation with normal donor PBLs (Jackson *et al,* 1988; Gallo *et al,* 1987; Castro *et al,* 1988). The separation of PBLs was done by Ficoll-Hypaque gradient from heparinized blood. T-cell subsets were analyzed by flow cytometry on a Coulter EPICS Profile (Coulter Electronics Co, Hialeah, FL).

RESULTS

By September 1990, 54 HIV seropositive women had been interviewed. The mean age was 26.4 years (range: 18-35 years). The probable mode of infection with HIV was heterosexual intercourse, since no history of IV drug use was noted and only one woman had received a blood transfusion, nine months before HIV infection was diagnosed.

Pregnancy course/outcome

Twenty-six of these women had a total of 37 pregnancies temporally related to their HIV infection. Eight were pregnant at the time of HIV diagnosis (Table 1). Three had term deliveries;

Table 1

Pregnancy outcome in HIV-1 seropositive women: 8 pregnant at the time of HIV diagnosis.

Outcome	Repeat pregnancies
2 Term	Term
1 Term	Induced abortion
1 Premature*	Pregnant**
2 Induced abortion	
1 Induced abortion	Induced abortion
1 Pregnant***	

*Died shortly after birth; not tested
**Lost to follow-up at four months.
***Lost to follow-up at three months.

one had a premature delivery at eight months; three had abortions; one pregnant woman was lost to follow-up. Five women in this group had repeat pregnancies: two term deliveries, and one lost to follow-up while pregnant.

Eighteen other women became pregnant after HIV diagnosis (Table 2), which resulted in seven term deliveries, one ectopic pregnancy which was terminated, and eight abortions. One woman is currently pregnant, and one woman was lost to follow-up while pregnant. Five women in this group also had repeat pregnancies: two premature deliveries at six months (one was a set of twins), and five abortions.

Table 2

Pregnancy outcome in HIV-1 seropositive women: 18 pregnant after HIV diagnosis.

Outcome	Repeat pregnancies
3 Term	
2 Term	Premature*
	Induced abortion
1 Term	Premature**
1 Term	Induced abortion
1 Term	2 Induced abortions
1 Ectopic pregnancy	
7 Induced abortion	
1 Induced abortion	Induced abortion
1 Pregnant	
1 Pregnant***	

* Died shortly after birth; not tested.
** Died shortly after birth (twins); cord bloods EIA and WB positive.
*** Lost to follow-up at two months.

Clinical/immunologic status of the mothers

The CDC Group Classification of most of the HIV-1 infected women that had full term or premature deliveries did not change during pregnancy; however, three women were reclassified from either CDC Groups II or III to Group IV C-2 because of the finding of the oral hairy leukoplakia (OHL). The CD4+ cell counts of the pregnant women did not show any trend suggestive of a consistent decrease associated with pregnancy (Table 3).

Clinical/serologic status of babies

Twelve term babies were born to HIV seropositive mothers, one of these infants died at three months of age. This baby had tested positive for HIV antibody by EIA at three weeks (Table 4). The clinical symptoms and status with respect to HIV infection at the time of death were not determined. The mean age of the 11 surviving term babies at the time of analysis was 17.7 ± 9.5 months. Non-specific findings have developed in most of these babies that are suggestive, but not diagnostic of HIV: persistent generalized lymphadenopathy in eight, developmental delay in two, oral candidiasis in one, and primary complex in three.

Three of these infants (case numbers 1-3) that have been followed for $\geq$ 20 months have sero-reverted from positive to negative for HIV-1 antibody, and have remained negative (Table 4). An accurate average time to sero-reversion cannot be determined because of the different intervals between follow-up visits. The serological status of two of the infants, however, had become indeterminant before 12 months, and both of these were non-reactive by 18 months of age. Infants 5 and 6 whose initial samples were indeterminant still remain in an indeterminant status after over two years of follow-up because of their WB banding patterns. Infant number 5 became completely negative at 13 and 15 months, but the EIA on the most recent test has become strongly positive. Even though the p24 band is not present on the WB for this sample, the gp120/160 band is strong. Infants 4, 9, 10 and 11 have not been monitored long enough to determine with certainty their HIV infection status at this time.

Table 3

CD4+ cell counts of HIV-1 positive women in relation to age of gestation*

case	Pregnancy outcome**	Pre-pregnancy	Trimester			Post-pregnancy
			1st	2nd	3rd	
RE	PM	356*** (− 4)****	120 (1)	320 (5)	ND	258 (+ 4)
MS	FT	734 (− 1)	457 (3)	1345 6)	ND	1126 (+ 5)
LS	PM	717 (− 1)	ND	1165 (4)	743 (7)	619 (+ 4)
RD	FT	821 (− 3)	876 (1)	479 (5)	566 (9)	617 (+ 3)
MG	FT	275 (− 5)	370 (2)	217 (5)	619 (9)	323 (+ 3)
GV	PM	621 (− 3)	522 (3)	707 (6)	ND	530 (+ 1)
ES	FT	348 (− 5)	ND	815 (4)	347 (9)	327 (+ 3)

* Women who had full term or premature deliveries and at least 2 CD4 + cell counts available during their pregnancy.
** FT = full term delivery; PM = premature delivery ($\geq$ 6 months age of gestation).
*** Cells/mm^3
**** ( ) = months pregnant; − denotes months prior to pregnancy; + denotes months after delivery.

Both infants 7 and 8 appear to be truly infected with HIV-1. After being negative at 12 months, infant 7 has developed a complete HIV-1 WB banding profile and reactivity to both recombinant proteins by the confirmatory competitive EIA in the 16-month sample. This most recent serum sample also was tested for p24 antigen and the PBLs were cultured for virus isolation with negative results. Infant number 8 has been consistently positive by the WB assay except for the last sample taken at 20 months of age which shows an indeterminant WB banding pattern with the absence of p24; however, the 17-month sample was positive by both p24 antigen capture EIA and virus culture. The 20-month sample was not tested for p24 antigen or cultured for virus isolation because of insufficient volume. Clinically these two babies have presented with persistent generalized lymphadenopathy, developmental delay and primary complex by chest x-ray examination.

DISCUSSION

This is the first documentation of perinatal HIV-1 transmission in the Philippines. Two out of 11 children born to HIV-1 infected women were diagnosed positive based on the persistence of antibody beyond 15 months of age in both cases as well as the detection of p24 antigen and virus isolation in one of the cases. While it is possible that these infants were not infected at birth but later in childhood through breastfeeding, this mechanism of transmission appears to be rare (Oxtoby, 1988). Also, all of the mothers in our study were provided free infant formula to minimize this possibility. Some of the remaining children in our study that are currently reactive for HIV-1 antibody by EIA and WB assay also may be infected, but they still have not been followed long enough to rule out the presence of passively acquired HIV-1 antibody from their mothers.

The diagnosis of HIV-1 infection in neonates by EIA and WB assay immunoreactivity is complicated by the presence of maternal antibody. These serological tests detect anti-HIV IgG antibody which crosses the placenta from the infected mother, and can persist in the infant for over a year. Because of this persistence of maternal antibody, the infant usually cannot be confirmed positive for HIV-1 infection until $\geq$ 15 months of age. By this time maternal antibody should have waned and *de novo* synthesis of antibody in the infected infant is expected to have occurred. To diagnose HIV-1 infection in infants < 15 months of age currently requires the detection of p24 antigen or the isolation of virus by co-culture.

Table 4

HIV-1 antibody status of full term babies born to HIV positive women.

Case no.	HIV antibody results Screening ELA/WB or confirmatory ELA* (age in months)
Alive	
1	+/+ (4, 8); −/± (11); −/− (14, 19, 22, 30, 33)
2	+/+ (2, 6); −/± (10); −/− (17, 20)
3	+/+ (7); −/− (21)
4	+/NT** (3); +/+ (5); −/− (12)
5	+/± (3, 6); −/± (9); −/− (13, 15); −/± (20); −/± (29)
6	+/± (5, 6); −/NT (15); −/± (23, 26)
7	−/NT (9); −/− (12); +/+ (16)
8	+/+ (3, 9, 12, 17); +/± (20)
9	+/+ (3, 6)
10	+/+ (3, 5)
11	+/+ (3, 7)
Died	
12	+/NT (< 1)

* For WB assay + = bands to p24 and gp41 and/or gp121/160 must be present; − = no bands present; ± = bands present but not to p24 and gp41 and/or gp120/160. For confirmatory EIA (ENVACOR) + = reactivity to both recombinant p24 and gp41 proteins; − = no reactivity to either protein; ± = reactivity to only one of the recombinant proteins.

** NT = not tested.

Unfortunately the latter technique can only be performed in a specialized laboratory, and p24 antigen may not be detectable if the infection is latent or in the presence of maternal HIV-1 antibody (CDC, 1987; Rakusan *et al,* 1991). Other tests that may be useful for early diagnosis such as the detection of IgM or IgA antibodies, which do not cross the placenta, and the polymerase chain reaction for the detection of viral nucleic acid are still in the developmental stage and remain to be standardized for routine laboratory use (Rakusan *et al,* 1991; Gaetano *et al,* 1987; Rogers *et al,* 1989).

One of the HIV-1 infected infants in our study seroconverted for HIV-1 antibody by both EIA and WB assay between 12 and 16 months of age. Apparently the maternal antibody had disappeared by 9 months of age, the earliest sample available, and *de novo* antibody synthesis did not occur until after 12 months of age. Delayed *de novo* synthesis of HIV-1 antibody has been reported to occur in up to 10% of infected infants (CDC, 1987; Rakusan *et al,* 1991; Borkowski *et al,* 1987). The frequency of occurrence of this phenomenon indicates that the infant (case #4) in our study that sero-reverted between 5 and 12 months of age still needs to be monitored closely for several more months before concluding that HIV-1 infection is absent. The two infants whose sera show an indeterminate WB HIV-1 protein banding pattern at 26 and 29 months of age, respectively, also will require additional follow-up to rule out HIV-1 infections. Even if these latter infants are negative, the rate of HIV-1 perinatal transmission observed in the group of infants followed for ≥ 15 months will be 2/7 (28.6%) which falls in the 25-60% range reported from other studies (MacDonald *et al,* 1991).

Whether or not pregnancy accelerates the progression of HIV-1 associated disease remains an unsettled issue (MacDonald, 1991). In this study three of our patients were classified into a higher CDC group during the course of the pregnancy because of the diagnosis of OHL; however, it is not clear if this was a new clinical manifestation in these patients. We were able to diagnose OHL for the first time in a number of other HIV-1 infected women in our study during this same period

probably because of an increased awareness of this disease. Pregnancy has been reported to increase the rate of decline in CD4+ cells in HIV-1 infected women (Gall, 1990; Biggar *et al,* 1989), but for the small number of pregnant women in our study that had an adequate number of CD4+ cell counts no consistent pattern of decline was observed.

Although to date the documented occurrence of perinatal HIV-1 transmission has been very infrequent in the Philippines, the potential for this problem to increase must remain a public health concern. Of the 54 HIV-1 positive women working as prostitutes who have enrolled in our prospective epidemiological studies since 1985, over two-thirds have reported at least one pregnancy prior to diagnosis (unpublished data). Even more disturbing is the fact that one-third of these women still became pregnant even after learning that they were infected with HIV-1 and being counseled on the risk of perinatal transmission. Follow-up studies must be continued to monitor the full extent of this evolving epidemic and to design more effective intervention strategies to reduce transmission.

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REFERENCES

Biggar RJ, Pahwa S, Minkoff H,*et al.* Immunosuppression in pregnant women infected with human immunodeficiency virus. *Am J Obstet Gynecol* 1989; 161 : 1239-44.

Borkowski W, Krasinski K, Paul D, Moore T, Beneroth D, Chandwani S. Human immunodeficiency virus infections in infants negative for anti-HIV by enzyme linked immunoassay. *Lancet* 1987; 1 : 1168-71.

Castro BA, Weiss CD, Wiviott LD, Levy JA. Optimal conditions for recovery of the human immunodeficiency virus from peripheral blood mononuclear cells. *J Clin Microbiol* 1988; 26 : 2371-4.

Centers for Disease Control. Revised of case definition for acquired immunodeficiency syndrome. *MMWR* 1987; 36 : 142-3.

Gaetano C, Scano G, Carbonari M, *et al.* Delayed and defective anti-HIV IgM response in infants. *Lancet* 1987; 1 : 631.

Gall SA. Human immunodeficiency virus infection in pregnancy. *Immunol Allerg Clin North Am* 1990; 10 : 133.

Gallo D, Kimpton JS, Dailey PJ. Comparative studies on use of fresh and frozen peripheral blood lymphocyte specimens for isolation of human immunodeficiency virus and effects of cells lysis on isolation efficiency. *J Clin Microbiol* 1987; 25 : 1291-4.

Hayes CG, Manaloto CR, Basaca-Sevilla V, *et al.* Epidemiology of HIV infection among prostitutes in the Philippines. *J Acquir Imm Defic Syndr* 1990; 3 : 913-20.

Jackson JB, Coombs RW, Sannerud H Jr, Rhame Balfour RR Jr. Rapid and sensitive viral culture method for human immunodeficiency virus type 1. *J Clin Microbiol* 1988; 26 : 1416-8.

MacDonald MG, Ginzburg HM, Bolan JC. HIV infection in pregnancy: Epidemiology and clinical management. *J Acquir Imm Defic Syndr* 1991; 4 : 100.

National AIDS Prevention and Control Program, Department of Health, Philippines, September 1990.

Oxtoby MJ. Human immunodeficiency virus and other viruses in human milk: placing the issues in broader perspective. *Pediatr Infect Dis* 1988; 7 : 1825.

Rakusan TA, Parrott RH, Sever JL. Limitations in the laboratory diagnosis of vertically acquired HIV infection. *J Acquir Imm Defic Syndr* 1991; 4 : 116.

Rogers MF, *et al.* Value of PCR-indicator of AIDS/HIV in infants. *N Engl J Med* 1989; 320 : 1649-54.