

Investigation of HIV in Amniotic Fluid from HIV-Infected Pregnant Women at Full Term

Boshishi K. F. Mohlala,¹ Timothy J. Tucker,^{2,3} Mitchell J. Besser,¹ Carolyn Williamson,² Jane Yeats,² Lynette Smit,² John Anthony,¹ and Adrian Puren⁴

¹Departments of Obstetrics and Gynaecology and ²Medical Virology, Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, and ³Medical Research Council, Cape Town, and ⁴National Institute for Communicable Diseases, Johannesburg, South Africa

Background. In the absence of interventions and breast-feeding, the in utero transmission rate of human immunodeficiency virus (HIV) is estimated to be 10%–15%, and the role that amniotic fluid (AF) plays in this is unclear.

Objectives. Levels of cytomegalovirus (CMV) in AF and levels of HIV-1 in AF, maternal blood, and fetal cord blood were assessed.

Study design. We enrolled 23 HIV-1–positive women with healthy, singleton pregnancies who underwent elective cesarean section (CS) at full term. The Roche Amplicor HIV-1 Monitor test (version 1.5) was used for determination of maternal plasma VLs. The NASBA Nuclisens assay was used for determination of VLs in other samples. To determine the feasibility of detecting viral infections in AF, CMV polymerase chain reaction DNA extraction was performed on the AF samples by use of the QIAamp DNA kit.

Results. HIV-1 RNA was not detected in either AF or fetal cord blood. CMV was detected in 4 AF samples. Maternal CD4⁺ T cell counts were 158–654 cells/mL (mean, 405 cells/mL). The maternal plasma VLs ranged from below detectable limits to 169,990 copies/mL (mean, 33,700 copies/mL).

Conclusions. In the absence of medical complications and before labor, AF collected during elective CS from women who had received either zidovudine or nevirapine during late-stage pregnancy was free of HIV.

The number of cases of HIV infection in children continue to escalate globally. The World Health Organization estimated that, in 2002, HIV-infected children accounted for ~10% of the infections in developing countries. Perinatal transmission ac-

counts for >90% of HIV infections in infants and children and is responsible for almost all new HIV infections in preadolescent children. Perinatal transmission is also responsible for >90% of pediatric AIDS cases in the United States [1].

In the absence of intervention, the rate of mother-to-child transmission (MTCT) is 15%–25% in Europe and the United States [2] and 25%–40% in Africa and Asia [3]. The differences in the rates may reflect differences in the prevalences of the cofactors involved in MTCT, such as obstetric practices and breast-feeding. It is estimated that up to 30% of MTCTs, with the exclusion of those due to breast-feeding, occur in utero, with the remainder occurring intrapartum [1]. The most-potent predictors of perinatal HIV transmission are maternal HIV-1 load (VL), severity of maternal disease [4, 5], prolonged rupture of the amniotic membranes, and mode of delivery [6]. Several studies have demonstrated that delivery by elective cesarean section (CS) [7–10] before rupture of the amniotic membranes or the onset of labor, as well as the administration of zidovudine or nevirapine during the peripartum period, reduce MTCT significantly [11–14].

The possible mechanisms responsible for vertical transmission during the peripartum period include transplacental microtransfusions of maternal blood into the fetal circulation during contractions, labor, and separation of the placenta before clamping of the umbilical cord [15]; ascending infection from the vagina through the cervix after rupture of the amniotic membranes, which infects the amniotic fluid (AF); and absorption of the virus through the infant's immature digestive tract. Microtrauma to the fetal skin during birth, with exposure to blood and infected cervicovaginal secretions, may also be responsible for transmission of the virus. It is also possible that fetal exposure to HIV-infected AF or to the cells that are found within AF, before rupture of the amniotic membranes, could be responsible for a proportion of vertical transmission in utero.

It is not known whether HIV is present in AF before rupture of the amniotic membranes. Only 1 research letter (from 1987, describing a single patient) with data relating to the presence of HIV in AF in a viable pregnancy has been published. This case report described an amniocentesis performed for evaluation of erythroblastosis fetalis in an HIV-positive woman at 32 weeks of gestation. HIV was detected in this patient by use of

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Correspondence: Dr. Timothy J. Tucker, c/o Medical Research Council, PO Box 19070, Tygerberg, 7505, South Africa (tim.tucker@telkomsa.net). Reprints will not be available from the authors.

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viral culture. Maternal blood contamination of the AF was not excluded [16]. This study preceded the availability of more-sensitive HIV nucleic acid amplification technologies, and there are no data derived by use of these more-sensitive techniques for detection and/or quantification of VL in AF. The present study aimed to investigate the possible role that HIV plays in AF in MTCT by determining whether HIV is detectable in AF from HIV-positive women undergoing elective CS at full term and, if so, to determine whether there is a correlation between the VL in AF and that in maternal blood.

Patients, materials, and methods. The study was approved by the University of Cape Town Ethics Committee. Women were recruited to the study during antenatal care at 30–36 weeks of gestation, at which time written, informed consent was obtained. Consenting HIV-positive women with healthy, singleton pregnancies who chose to undergo elective CS at 38–40 weeks of gestation were enrolled. Women with other medical or obstetric complications were excluded, as were women who presented while in labor. Women were admitted to the study hospital the night before their scheduled elective CS. The women either received a single dose of nevirapine (200 mg) at least 4 h before undergoing surgery or continued to receive a regimen of oral zidovudine tablets (300 mg) twice daily (which began at 34 weeks of gestation) and received a single dose of zidovudine (300 mg) at least 3 h before undergoing surgery.

Elective CSs proceeded with routine celiotomy and transverse incision of the lower uterine segment. The amniotic membranes were exposed to the external uterine environment but not ruptured. The amniotic membranes were cleaned with large volumes of saline and sterile gauze swabs. An 18-gauge needle attached to a 20-mL syringe was used to pierce the amniotic membranes and to collect AF. This occurred before surgical incision of the amniotic membranes. After collection of AF, the CS was completed routinely. Samples of AF grossly contaminated with meconium or blood were excluded from the analysis. Placental bed biopsy specimens were obtained after delivery. Women were excluded if there were any macroscopic signs of placental abnormalities, such as hemorrhage, thrombosis, infarction, meconium, or mass.

The biological samples were obtained during surgery, and all of the women were already receiving antiretroviral therapy. The following samples were obtained: AF, maternal blood, fetal cord blood, and placental bed biopsy specimens. Samples for VL analysis were processed and, within 4 h, were stored at -70°C . Maternal HIV diagnoses were confirmed by use of routine HIV ELISA antibody tests (Abbott AxSYM). The Roche Amplicor HIV-1 Monitor test (version 1.5; Roche Diagnostics) was used for quantification of maternal plasma VL by use of 200 μL of plasma for RNA extraction. The NASBA Nuclisens assay (Organon Teknica) was used for quantification of AF VL and fetal cord blood VL by use of 200 μL of fetal cord blood plasma

and 2 mL of AF for RNA extraction. The VL sensitivity for assessment of AF was $\sim 25\text{--}90$ copies/mL. So that potentially HIV-infected cellular material could also be detected, AF samples were not spun before RNA extraction.

For the detection of cytomegalovirus (CMV), DNA extraction was performed on 200 μL of each AF sample by use of the QIAamp DNA Mini Kit (QIAGEN), in accordance with the manufacturer's protocol. Ten microliters of DNA eluate was included in a 50- μL PCR reaction using standard PCR reagents and reagent concentrations and previously published CMV primers that target the immediate-early antigen region and yield a 160-bp product [17]. PCR products were visualized under UV light after agarose gel electrophoresis and ethidium bromide staining. The 23 AF samples were tested as a single batch, and 2 negative controls and 1 positive control were included. CMV-positive samples underwent repeated amplification to confirm the results.

Placental bed biopsy specimens were fixed overnight in 10% formalin and set in paraffin wax, and 5- μm sections were cut and placed on aminopropyl triethoxysilane-coated slides and, after routine hematoxylin-eosin staining, were examined for signs of inflammation or other pathological abnormalities. CD4⁺ T, CD8⁺ T, full blood, and differential white cell counts were performed by routine analysis. AF was assessed for the presence of maternal and/or fetal blood by use of the Kleihauer-Betke test. All blood-contaminated AF samples were excluded from the analysis. The protocol followed in the present study was approved by the University of Cape Town Ethics Committee on Human Experimentation.

Results. Twenty-six HIV-positive women were recruited into the study. The mean age of the women was 27.9 years (range, 17–35 years) (the ages of 3 of the women were not documented). None of the women had pregnancy-related complications other than HIV infection. Eleven women received nevirapine therapy, and 12 received zidovudine therapy. HIV was not detected in any of the AF samples. CMV was detected in 4 samples. The maternal plasma VLs ranged from lower than detectable limits (<400 copies/mL) to 169,990 copies/mL (mean, 33,700 copies/mL) (table 1). The maternal CD4⁺ T cell counts ranged from 158 to 654 cells/mL (mean, 405 cells/mL); 2 of the women suffered absolute lymphopenia. For all 23 fetuses, fetal cord blood samples obtained at birth were negative for HIV RNA. Placental biopsies showed no macroscopic abnormalities at the time of CS, and no inflammation or pathological abnormality was detected by microscopy. Three patient sample sets were excluded from analysis because of gross contamination with meconium and/or blood. By use of the Kleihauer-Betke test, none of the other samples was found to be positive for occult contamination with fetal blood. No sample was excluded from the analysis because of macroscopic placental abnormalities.

Discussion. Our findings demonstrate that the AF from

Table 1. Characteristics of the study patients.

Patient	Age, years	Zidovudine therapy	Nevirapine therapy	AF VL	Maternal plasma VL, copies/mL	Fetal cord blood VL	CD4 ⁺ T cell count, cells/mL	CD8 ⁺ T cell count, cells/mL	CD4 ⁺ T cell:CD8 ⁺ T cell ratio	CMV PCR AF
C10	Unknown	No	Yes	LDL	13,056	LDL	263	971	0.27	Neg
C11	Unknown	No	Yes	LDL	12,212	LDL	471	1250	0.38	Neg
C12	Unknown	No	Yes	LDL	11,110	LDL	562	716	0.78	Neg
C13	35	Yes	No	LDL	11,164	LDL	471	775	0.61	Neg
C14	25	Yes	No	LDL	168,066	LDL	372	454	0.82	Neg
C15	17	Yes	No	LDL	30,549	LDL	188	391	0.48	Neg
C16	25	Yes	No	LDL	86,910	LDL	372	634	0.59	Neg
C17	34	Yes	No	LDL	LDL	LDL	620	499	1.24	Neg
C18	33	No	Yes	LDL	LDL	LDL	617	1038	0.59	Neg
C19	28	Yes	No	LDL	5682	LDL	499	898	0.56	Neg
C20	24	No	Yes	LDL	882	LDL	568	777	0.73	Neg
C21	30	No	Yes	LDL	14,999	LDL	654	999	0.65	Neg
C22	30	No	Yes	LDL	1609	LDL	198	807	0.25	Neg
C23	34	No	Yes	LDL	169,990	LDL	160	1375	0.12	Pos
C24	29	No	Yes	LDL	30,646	LDL	582	829	0.70	Neg
C25	23	Yes	No	LDL	8010	LDL	236	423	0.56	Pos
C26	21	Yes	No	LDL	9216	LDL	565	585	0.97	Pos
C27	20	No	Yes	LDL	3814	LDL	477	1477	0.32	Neg
C28	29	No	Yes	LDL	24,369	LDL	417	675	0.62	Neg
C29	35	Yes	No	LDL	62,888	LDL	271	484	0.56	Pos
C30	26	Yes	No	LDL	55,231	LDL	158	1354	0.12	Neg
C31	29	Yes	No	LDL	6095	LDL	381	1375	0.28	Neg
C32	30	Yes	No	LDL	48,591	LDL	222	2029	0.11	Neg

NOTE. The median maternal plasma VL was 13,056 copies/mL, and the mean maternal plasma VL was 33,700 copies/mL. AF, amniotic fluid; LDL, less than the lowest detectable level; neg, negative; pos, positive; VL, HIV-1 load.

women with healthy, singleton pregnancies who underwent elective CS before labor, at 38–40 weeks of gestation, and who received a single dose of nevirapine or a short course of zidovudine was free of HIV. Although the exact mode of HIV transmission in utero remains speculative, our data suggest that, in normal, uncomplicated pregnancies, before the onset of labor or rupture of the amniotic membranes, the AF is free of HIV. Thus, the fetus is not exposed to HIV-infected AF before the onset of labor and/or rupture of the amniotic membranes.

In the present study, all 23 fetal cord blood VLs were less than the lowest detectable level, which implies that none of the 23 fetuses was infected in utero. Therefore, we can also conclude that, when a fetus is not infected with HIV, the AF is free of HIV. However, this might be different with an infected fetus, irrespective of the mode of infection. The upper bound of the 95% confidence interval for potential detection of HIV in the AF is 12%, given the negative results for the 23 women in the study.

The NASBA Nuclisens assay is accredited for detection of virus in biological fluids other than plasma. This extraction and amplification methodology allows for the quantification of a negligible number of HIV genome copies; the sensitivity of the assay used in the present study was ~40 HIV copies/mL. This sensitivity contrasts with that of viral culture used by Mundy

et al. [16]; by that method, VLs <1000 copies/mL are not normally detectable. The fact that no virus was found strengthens the argument that no HIV was present. This would include both free virus and intracellular virus, since samples were not centrifuged before genome extraction. To establish whether any virus could be isolated from these AF samples, the CMV PCR was performed on identical samples. This PCR isolated CMV and showed that virus genomes could be extracted and detected with ease in these AF samples.

None of the 23 fetuses was infected before birth, as shown by the lack of detectable virus in fetal cord blood. The relative risk of transmission to these fetuses at birth would have been lower than that in those of mothers without access to antiretroviral therapy and/or elective CS. However, this assessment at birth would reflect only established intrauterine infection. Vaginal delivery is associated with increased risk of MTCT; this increased risk is ascribed to increased exposure to infected genital secretions and microtrauma during birth [7–10]. The AF samples examined in the present study were obtained from women at 38–40 weeks of gestation, during elective CS and before labor. They thus represent samples from women who were at a stage of pregnancy before the cascade of inflammatory mechanisms that have been proposed to play a role in the initiation of parturition. The onset of labor is heralded by an

increase in proinflammatory agents, such as cytokines and prostaglandins [18–20]; therefore, the lack of detection of virus in AF may not hold true for vaginal deliveries. Vaginal delivery follows softening and dilatation of the cervix in an inflammatory-mediated process [21–23], which is followed by forceful contractions of the uterus that allow the delivery of the fetus. Changes in AF may occur because of the local inflammatory changes induced by labor and/or as the cervix effaces the amniotic membranes. It is possible that, in vaginal delivery, the greatest risk to the fetus is the effect of these inflammatory mediators. This highlights the need to assess the presence or absence of HIV in AF from women who are HIV positive but who make an informed choice to deliver vaginally.

We conclude that, in the absence of medical or obstetric complications and before labor, the AF from women who receive zidovudine or nevirapine during late-stage pregnancy will be free of HIV. These findings may not be true for complicated pregnancies and/or during labor and should be seen only as reflecting the situation in women before the onset of labor and at elective CS. Further investigation of women who have made an informed decision to deliver vaginally may show that AF contains HIV, which will increase the risk of perinatal transmission.

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