

## HIV-1 RNA detection in the amniotic fluid of HIV-infected pregnant women

Ana Christina Lobato<sup>1,2</sup>, Regina Amélia Aguiar<sup>2,3</sup>, Agdemir Waléria Aleixo<sup>2</sup>,  
Beatriz Amélia Andrade<sup>2</sup>, Ines Katherina Cavallo<sup>1,2</sup>, Fabiana Maria Kakehasi<sup>1,2</sup>,  
Jorge Andrade Pinto<sup>2,3</sup>, Victor Hugo Melo<sup>2,3/+</sup>

<sup>1</sup>Hospital das Clínicas <sup>2</sup>Grupo de AIDS Materno-Infantil <sup>3</sup>Faculdade de Medicina,  
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

*This study is aimed at evaluating the potential to detect human immunodeficiency virus (HIV) in amniotic fluid (AF) collected at delivery from 40 HIV-positive pregnant women. Thirty patients had a plasma viral load (VL) below 1,000 copies/mL at delivery. VL was positive in three AF samples. No significant association was found between the HIV-1 RNA in AF and the maternal plasma samples. There was no HIV vertical transmission detected.*

Key words: HIV infection - infectious disease transmission - vertical - amniotic fluid - viral load

The peak incidence of mother-to-child transmission (MTCT) for human immunodeficiency virus (HIV) occurs during labor, delivery and/or postnatally through breastfeeding (Magder et al. 2005). Over 35% of fetuses may be infected *in utero*, especially during the last weeks of pregnancy (Bertolli et al. 1996). Transplacental microtransfusions that occur during labor contractions, delivery, or placental separation play an important role in the intrapartum transmission of HIV (Lin et al. 1996). Other risk factors include ruptured membranes and fetal skin disruption during delivery (Cotter et al. 2004).

HIV is present in other bodily fluids such as sperm, vaginal discharge and maternal milk. It has also been detected in small quantities in saliva and tears. Contamination of the amniotic fluid (AF), or its cells, may explain MTCT prior to membrane rupture (Mohlala et al. 2005). The AF contains amniotic and fetal cells, although evidence that these cells may be infected with HIV is weak.

The presence of HIV in AF, or its cells, has been studied since 1987 (Mundy et al. 1987, Maiques et al. 2003, Mohlala et al. 2005), but HIV has only been detected in one study (Mundy 1987), where it was found in an AF sample obtained by amniocentesis from one patient. This was possibly due to contamination by the mother's blood. Other studies have looked at AF obtained by amniocentesis (n = 29) and at delivery during a c-section (n = 38) (Maiques et al. 2003) as well as during elective c-section at full term (n = 23) (Mohlala et al. 2005). Both studies failed to detect HIV in AF samples.

Viral cultures and genetic amplification of HIV are procedures that may help estimate when the MTCT occurred. Nucleic acid sequence-based amplification (NASBA) is one of the techniques employed to detect HIV in biological fluids (Mofenson 1997, Shepard et al. 2000).

The objective of this study was to test for the presence of HIV in AF collected from HIV-infected pregnant women at delivery and to compare the AF viral load (VL) between maternal and newborn plasma.

Forty-HIV-infected pregnant women consecutively admitted for elective c-section (there was no labor or rupture of membranes) at the Clinics Hospital, Federal University of Minas Gerais, Brazil, from 2005-2007, were enrolled in this study. The study was approved by the Institutional Research Ethical Committee and all participants provided written consent.

C-sections were performed using standard techniques until the moment of uterine incision, which was delicately executed so that the membranes remained intact. Membranes were punctured using an 18-gauge needle attached to a 20 mL syringe and at least 2 mL of AF were collected. Fluid was assessed macroscopically and samples contaminated by blood or meconium were discarded. Samples were immediately stored at -70°C.

NASBA and NucliSens® easyMAG™ (bioMérieux) methods were used to isolate, amplify and quantify HIV-1 RNA from AF samples. This method detects viral RNA in real time (sensitivity: 50%; 3 x 10<sup>6</sup> UI and specificity: 99.7%). Maternal and neonatal samples were collected immediately after delivery to determine the plasma VL. Uninfected infants were defined as those who tested negative in two separate plasma VL assays performed at four weeks old or those who seroreverted (a negative serologic test performed after 12 months of age) (Kakehasi et al. 2008).

During prenatal care, 37 (92.5%) of the women used the highly active antiretroviral therapy (HAART). Three women did not receive antiretroviral therapy: two of whom had delayed the onset of prenatal care and one who was diagnosed near delivery. VL was not detected in the AF of any of the untreated patients. All women received intrapartum intravenous zidovudine. Severe immunosuppression, defined as a lymphocyte T CD4<sup>+</sup> cell count below 200/mm<sup>3</sup>, was observed in a minority of the women studied: 16.7% during pregnancy and 13.9%

+ Corresponding author: victormelo@terra.com.br

Received 7 January 2010

Accepted 29 April 2010

after delivery. Thirty (75%) patients had a VL below 1,000 copies/mL at delivery and 21 had an undetectable VL. HIV vertical transmission did not occur among the mother-infant pairs enrolled in this study.

HIV was detected in three (7.5%) of the AF samples. The VL found in these samples was 100 UI/mL, 90 UI/mL and 58 UI/mL. The onset of prenatal care for all three women occurred in the second trimester of pregnancy. All three were on HAART, with an undetectable plasma VL during the antenatal, peripartum and post-partum periods. The VL was undetectable in these three neonates and all of them born after 38 weeks of gestation and weighed more than 2.500 g.

We were unable to identify a specific factor associated with HIV in the AF of these women. Nevertheless, HIV was not detected in the AF of patients with known risk factors for vertical transmission, such as high VL during pregnancy or the peripartum period, preterm birth or low birth weight (< 2.500 g). This suggests that the presence of HIV in AF is not necessarily related to fetal infection.

In fact, the absence of vertical transmission in this study group is likely due to the fact that most of these women received adequate prenatal care, used anti-retrovirals, and did not breastfeed. In addition, all of these patients underwent elective c-section - a known protective procedure. These results are in agreement with our previous report that showed a decrease of HIV vertical transmission (from 20-3%) during a seven-year period (1998-2005) among pregnant women who used these prophylactic procedures. In this study, vaginal delivery was associated with an increased risk for MTCT (odds ratio: 3,3; 95% confidence interval: 1,8-5,8) (Kakehasi et al. 2008).

We found no association between the presence of HIV in AF with maternal VL, severe maternal immunosuppression and the time when antiretrovirals were initiated during prenatal care. Furthermore, no correlation was found between AF VL and neonatal plasma VL and low birth weight or preterm infants. Our method of recruitment did not affect our results because women were successively enrolled without specific selection criteria.

All women with detectable HIV in their AF delivered non-infected neonates. It may be that the HIV present in their AF was in its free form, since cells commonly infected by HIV are not present in AF. In contrast to previous research (Best 1996), we did not observe any correlation between HIV in AF and fetal infection.

Using polymerase chain reaction, Mundy et al. (1987) detected HIV in AF obtained by amniocentesis from an HIV-infected pregnant woman with Rh alloimmuniza-

tion. However, Maiques et al. (2003) and Mohlala et al. (2005), using the NASBA method, failed to detect HIV in AF. It is possible that the HIV found in AF has been a consequence of microscopic contamination during membrane puncture or fluid aspiration.

Our study demonstrates that it is possible to detect HIV in AF. Neither the mechanisms of HIV contamination of the amniotic cavity nor the risks of fetal infection in such cases have been established.

## REFERENCES

- Bertolli J, St Louis ME, Simonds RJ, Nieburg P, Kamenga M, Brown C, Tarande M, Quinn T, Ou CY 1996. Estimating the timing of mother-to-child transmission of human immunodeficiency virus in a breast-feeding population in Kinshasa, Zaire. *J Infect Dis* 174: 722-726.
- Best JM 1996. Laboratory diagnosis of intrauterine and perinatal virus infections. *Clin Diagn Virol* 5: 121-129.
- Cotter A, Duthely ML, Gonzalez-Quintero V, Sullivan MJO 2004. Duration of membrane rupture and vertical transmission of HIV: does the four hour rule still apply? *Am J Obstet Gynecol* 191 (Suppl. 1): S56.
- Kakehasi FM, Pinto JA, Romanelli RMC, Carneiro M, Cardoso CS, Tavares MCT, Melo VH, Aguiar RALP 2008. Determinants and trends in perinatal human immunodeficiency virus type 1 (HIV-1) transmission in the metropolitan area of Belo Horizonte, Brazil: 1998-2005. *Mem Inst Oswaldo Cruz* 103: 351-357.
- Lin HH, Kao JH, Hsu HY, Mizokami M, Hirano K, Chen DS 1996. Least microtransfusion from mother to fetus in elective cesarean delivery. *Obstet Gynecol* 87: 244-248.
- Magder LS, Mofenson L, Paul ME, Zorrilla CD, Blattner WA, Tuomala RE, LaRussa P, Landesman S, Rich KC 2005. Risk factors for in utero and intrapartum transmission of HIV. *J Acquir Immune Defic Syndr* 38: 87-95.
- Maiques V, García-Tejedor A, Perales A, Córdoba J, Esteban RJ 2003. HIV detection in amniotic fluid samples. Amniocentesis can be performed in HIV pregnant women? *Eur J Obstet Gynecol Reprod Biol* 108: 137-141.
- Mofenson LM 1997. Mother-child HIV-1 transmission: timing and determinants. *Obstet Gynecol Clin North Am* 24: 759-784.
- Mohlala BK, Tucker TJ, Besser MJ, Williamson C, Yeats J, Smit L, Anthony J, Puren A 2005. Investigation of HIV in amniotic fluid from HIV-infected pregnant women at full term. *J Infect Dis* 192: 488-491.
- Mundy DC, Schinazi RF, Gerber AR, Nahmias AJ, Randall HW Jr 1987. Human immunodeficiency virus isolated from amniotic fluid. *Lancet* 2: 459-460.
- Shepard RN, Schock J, Robertson K, Shugars DC, Dyer J, Vernazza P, Hall C, Cohen MS, Fiscus SA 2000. Quantitation of human immunodeficiency virus type 1 RNA in different biological compartments. *J Clin Microbiol* 38: 1414-1418.