Intrapartum Mucosal Exposure to Human Immunodeficiency Virus Type 1 (HIV-1) of Infants Born to HIV-1–Infected Mothers Correlates with Maternal Plasma Virus Burden


The majority of vertical infections with human immunodeficiency virus type 1 (HIV-1) occur at or near delivery, strongly suggesting a mucosal route of transmission. The frequency and level of intrapartum mucosal exposure to HIV-1 of 22 infants born to infected mothers was investigated. Maternal plasma HIV-1 RNA and CD4 cell count were measured at delivery. Infant oropharyngeal aspirates obtained at birth were examined for HIV-1 RNA by reverse transcription–polymerase chain reaction and qualitative nucleic acid sequence–based amplification. Nine infants (41%) had detectable levels of HIV-1 RNA, 3 of which were quantifiable (mean, 3000 copies/mL). This mucosal exposure to HIV-1 during delivery did not lead to infection of any infant. Cesarian delivery did not reduce mucosal exposure to HIV-1. Mucosal exposure did not correlate with maternal CD4 cell count but did correlate with maternal plasma virus load and was reduced by antiretroviral therapy.

Materials and Methods

Study design. The level of HIV-1 RNA in OPA of neonates born to HIV-1–infected women receiving prenatal care at St. Mary’s Hospital, London, was analyzed prospectively. Mothers were recruited into the study antenatally and their clinical, laboratory, and therapeutic profiles collected. Twenty of the 23 mothers were of African origin and the median age of the cohort was 28 years (range, 20–35). All infants were followed prospectively from birth, and HIV-1 infection status was determined by viral culture and PCR.

OPA. OPA were collected by vacuum suction of the neonates’ mouths immediately after birth. The volumes of OPA collected...
ranged from 200 μL to 2 mL. Within 2 h of collection, OPA were equally divided into 100-μL aliquots and centrifuged at 12,000 g for 5 min at room temperature. Electron microscopy studies have shown that HIV-1 particles form aggregates in adult human salivary mucins [9]. OPA were therefore centrifuged after collection, and HIV-1 RNA was assessed in both supernatants and pellets. The supernatants and pellets were either stored at −70°C until used or immediately processed for RNA extraction using the nucleic acid sequence-based amplification (NASBA; Organon Teknika, Boxtel, The Netherlands) method according to the manufacturer’s instructions. For both NASBA and reverse transcription (RT) PCR amplification, low positive (100 HIV-1 RNA copies/100 μL) and negative materials extracted in parallel with the clinical specimens were used as controls.

**HIV-1 RNA detection.** HIV-1 RNA amplification was carried out using both an in-house RT-PCR and the NASBA assay. All OPA supernatants and pellets positive in the NASBA HIV-1 RNA qualitative assay and all maternal plasma samples collected at delivery were subsequently quantified using the quantitative NASBA assay (calibrators diluted 1/10). This assay has a threshold detection limit of 1300 RNA copies/mL.

The RT-PCR was carried out using nested primer sets based on conserved regions of the HIV-1 pol gene and are as follows (relative to HIV-1Lai): outer sense, 5′-CATGGGTACACGACACAAAAAG (nt 4165−4186); outer anti-sense, 5′-TCTACTGGTTCCAGGCACTG-3′ (nt 4386−4408); inner sense, 5′-AAAGGAATTTGGAGGAG-TATTTGGAAAGTAG (nt 4182−4209); and inner anti-sense, 5′-CAGCAGTGGCTCTCAATTC (nt 4294−4317) (137-bp product).

Extracted RNA (5 μL) was subjected to cDNA synthesis for 1 h at 42°C in a reaction volume of 20 μL containing 6 μM outer anti-sense primer, 1× first-strand buffer (Gibco-BRL, Gaithersburg, MD), 20 U of RNasine (Promega, Madison, WI), 10 mM dithiothreitol (Gibco-BRL), each dNTP at 500 μM (Pharmacia, Milton Keynes, UK), and 100 U of reverse transcriptase (MLV-RT Superscript; Gibco-BRL). cDNA (5 μL) was used for the nested PCR, which was carried out in quadruplicate. The first-round PCR mixture contained 1× buffer II (Perkin Elmer, Norwalk, CT), 1.5 mM MgCl2, each dNTP at 30 μM, 10 pmol of each outer primer, and 2 U of Taq polymerase (Perkin Elmer). First-round amplification product (1.5 μL) was nested in a second-round PCR (20 μL) carried out under the same conditions as the outer PCR but containing 5 pmol of each inner primer. The cycling conditions for the first round were 94°C for 30 s, 45°C for 42 s, 72°C for 42 s (30 cycles), and a final extension at 72°C for 10 min. For the second round, the annealing temperature was increased to 52°C. Amplification reactions were carried out on a thermocycler (model 480; Perkin Elmer). The nested amplification products (5 μL) were visualized on a 2% agarose gel by ethidium bromide staining.

**Lower detection limit of HIV-1 RNA by amplification using OPA.** All RNA isolation was carried out using the NASBA assay, which includes an RNA internal control in each extraction tube. Amplification of this control acts as an indicator of the extraction efficiency and a marker for the presence of potential inhibitors in the clinical specimen.

HIV-1-negative OPA of different consistencies that fell into three categories (blood-stained, meconium-stained, and clear) were spiked with a fixed number of HIV-1 RNA molecules and used in control experiments to define the lower detection limits of both the qualitative NASBA and RT-PCR assays. Extractions and amplifications of the three categories of OPA with each dilution (100, 50, and 25 copies/100 μL) were carried out in triplicate to determine whether the quality of the OPA affects the sensitivity of the assays.

**Statistical analysis.** Medians were compared by Mann-Whitney U test, and the proportions of patients in distinct groups were compared using Fisher’s exact test.

**Results**

**Study population.** The route of maternal infection was presumed to be heterosexual, with the exception of 2 mothers of Caucasian origin who had used intravenous drugs. Only 1 mother had AIDS, the remaining being asymptomatic. Seven mothers previously had HIV-infected children and 6 mothers were primiparous. Twelve mothers had received zidovudine monotherapy according to the AIDS Clinical Trials Group 076 protocol [10], and 4 mothers received zidovudine and lamivudine. One mother-infant pair was excluded from further analysis because the maternal viral variant could not be detected by NASBA or RT-PCR. Subtyping of this HIV-1 strain by heteroduplex mobility assay [11] showed this variant to be of subtype G. None of the 22 infants were HIV-infected.

**HIV-1 RNA detection in OPA.** For OPA, the lower detection limit of the NASBA is 25−50 HIV-1 RNA copies/100 μL compared with 50−100 copies/100 μL in RT-PCR. In NASBA of samples spiked with 50 and 25 copies, RNA was detected in 100% and 55% of cases, respectively. In RT-PCR of samples spiked with 100, 50, and 25 copies, RNA was detected in 100%, 78%, and 22%, respectively, regardless of the nature or appearance of the OPA.

HIV-1 RNA was detected in the OPA of 9 (41%) of the 22 infants. Two OPA were positive for HIV-1 RNA only in the pellet fraction, 4 were positive only in the supernatant, and 3 were positive in both fractions. Three of the 9 positive OPA were negative by RT-PCR, perhaps due to its lower sensitivity. Four of the 22 pellets tested were inhibitory to the NASBA assay, as the internal controls could not be detected; this is probably due to excess protein overloading the silica matrix during the RNA isolation. The discrepant results between supernatants and pellets might reflect the variable constitution of clinical specimens such as OPA, with more viscous and proteinaceous OPA confining virus particles to the pellet fraction by aggregation and entrapment.

Three OPA (2 supernatants, 1 pellet) were quantifiable by NASBA with loads of 4100, 2730, and 2333 HIV RNA copies/mL. Two of these quantifiable OPA corresponded to the mothers with high virus load at delivery (130,000 and 57,000 HIV RNA copies/mL). It is important to note that 7 of 9 infants with detectable HIV-1 RNA in OPA were delivered by elective cesarian section, which suggests that the source of virus was maternal blood rather than maternal cervicovaginal secretions, as these infants did not come into contact with the birth canal. At these levels of mucosal exposure to the maternal virus, none of the 9 infants proved to be infected.
Table 1. Detection of HIV-1 RNA in oropharyngeal aspirates (OPA) of neonates and maternal correlates.

<table>
<thead>
<tr>
<th>Maternal factors</th>
<th>HIV-1 RNA in infant OPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n = 9)</td>
</tr>
<tr>
<td>CD4 cell count × 10^6/L*</td>
<td>275 (153–380)</td>
</tr>
<tr>
<td>Spontaneous vaginal delivery</td>
<td>2/9</td>
</tr>
<tr>
<td>Delivery with labor³</td>
<td>2/9</td>
</tr>
<tr>
<td>Perinatal antiretroviral therapy</td>
<td>4/9</td>
</tr>
<tr>
<td>Plasma virus load, log_{10} RNA copies/mL</td>
<td>4 (2.9–5.1)</td>
</tr>
</tbody>
</table>

NOTE. Nos. shown for CD4 cell count and plasma virus load from maternal samples obtained at delivery are median (range).

* One CD4 cell count from HIV-1-positive OPA group was not available.
² Mann-Whitney U test.
³ Fisher’s exact test.
§ Spontaneous vaginal deliveries and emergency cesarian sections.

**Maternal and obstetric factors.** The median maternal plasma virus load at delivery was significantly higher for the group in whom infant OPA was HIV RNA-positive (4.0 log_{10} RNA copies/mL) than for the group in whom neonate OPA was HIV RNA-negative (3.1 log_{10} RNA copies/mL) (Mann-Whitney U test, P < .05). Antiretroviral therapy significantly reduced the incidence of mucosal exposure; 4 of the 9 mothers of infants with positive OPA had received antiretrovirals, compared with 12 of 13 mothers whose infants had negative OPA (Fisher’s exact test, P = .023). There was no correlation between OPA positivity and mode of delivery or median maternal CD4 cell count at delivery. Table 1 summarizes the relationships between an HIV-1-positive OPA and maternal factors.

**Discussion**

The presence in 22 neonates of HIV-1 RNA in OPA obtained immediately at delivery was used as a marker of mucosal exposure to HIV-1 during delivery. We detected HIV-1 RNA in the OPA of 41% (n = 9) of infants born to HIV-1–infected women. The virus load was low, with 6 of the 9 having levels below the threshold of the NASBA quantitative assay (1300 HIV RNA copies/mL) and a mean load of 3000 HIV RNA copies/mL in the remaining 3. This level of exposure did not result in infection of the infants. This observation is in accordance with the SIV model where only high virus titers induce infection of newborn monkeys via the oral route [7].

The fact that none of the 22 infants was found to be infected may be due to the relatively low maternal virus burden of this cohort and the widespread use of perinatal antiretroviral therapy (16/22). Detection of HIV-1 RNA in the mouth of neonates immediately after delivery correlated with maternal virus load. Previous studies have highlighted the lack of direct correlation between peripheral virus load and CVS viral shedding [4, 12], so correlation analysis could be confounded by the absence of virus load measurement in the CVS of these women. However, in our study, 7 of 9 infants with HIV-positive OPA were delivered by elective cesarian section, which suggests that in those cases, the source of virus was maternal blood.

Our pilot study shows that virus can be found in >40% of neonates’ OPA obtained at delivery. Intrapartum exposure to a detectable level correlated with maternal plasma HIV burden, and perinatal antiretroviral therapy was significantly associated with a reduced incidence of oral exposure to HIV-1. For countries where antiretroviral therapy is not affordable, development of birth canal preventative interventions is important. Biggar et al. [13] undertook such a study in Malawi, using vaginal cleansing with chlorhexidine solution every 4 h during labor. Overall, there was no difference in the rate of HIV transmission between the intervention and control groups (transmission rate with chlorhexidine, 27%; without chlorhexidine, 28%). However, when the fetal membranes were ruptured for ≥4 h, vaginal cleansing appeared to have an effect (transmission rate with chlorhexidine, 25%; without chlorhexidine, 39%; P = .02). Although further studies will be necessary to correlate the detection of HIV-1 RNA in OPA with a higher risk of transmission, our technique could be used to assess the effect of birth canal interventions designed to prevent perinatal transmission.

**Acknowledgments**

We thank the mothers and their infants who took part in this study; St. Mary’s Hospital Trust midwives, S. Dick and M. Horby, for their assistance with the management of the study; and R. Pitman for providing the primers for the HIV-1 pol RT-PCR.

**References**


Human Immunodeficiency Virus Type 1 RNA Shedding in the Female Genital Tract

Claudia Goulston,* William McFarland, and David Katzenstein

Cervical and plasma samples obtained twice, at 2-week intervals, from 49 human immunodeficiency virus type 1 (HIV-1)–positive women were assayed for HIV-1 RNA. More than 100 copies of HIV-1 RNA were detected in cervical swab supernatants (CSS) from 24 (49%) of 49 women. HIV-1 RNA in CSS was detected in younger women with higher levels of plasma HIV-1 RNA (median, 31,984 vs. 2880 copies/mL; \( P = .0004 \)), lower CD4 cell counts (median, 190 vs. 390 per \( \mu \)L; \( P = .012 \)), and lower CD4 cell percents (median, 16% vs. 25%; \( P = .03 \)). In multiple logistic regression analysis, only plasma HIV-1 RNA was significantly associated with CSS HIV-1 RNA, with an odds ratio of 4.79/log10 increase in plasma HIV-1 RNA (95% confidence interval, 1.4–16; \( P = .01 \)). Detection of HIV-1 RNA in cervical secretions is primarily associated with increased plasma HIV-1 RNA.

The contributions of human immunodeficiency virus type 1 (HIV-1)–infected cells and cell-free virus in the genital tract to sexual and vertical transmission of HIV-1 are not fully known. Studies of female genital tract secretions have identified the presence of HIV-1 by culture and polymerase chain reaction (PCR) amplification of HIV-1 proviral DNA [1–8]. Detection of cell-associated HIV-1 DNA in the genital tract has been associated with oral contraceptive use, cervical mucopus and inflammation, and genital ulceration [4–6]. Cell-free virus has been detected in genital tract secretions in 20%–50% of women by use of an internally controlled RT-PCR assay to quantify HIV-1 RNA [14]. Women were studied at two time points to determine if there were differences in plasma...