HIV-1 vertical transmission in Rio Grande, Southern Brazil

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ORIGINAL RESEARCH ARTICLE

HIV-1 vertical transmission in Rio Grande, Southern Brazil

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Summary: The aim of this study was to determine the rate and risk factors of HIV-1 mother-to-child transmission (MTCT), the timing of transmission and the transmitted subtype in a population where subtypes B and C co-circulates. One hundred and forty-four babies born to HIV-1-infected mothers were studied. Subtype and timing of transmission was determined by a nested polymerase chain reaction of gp41 gene. Seven children were infected (4.9%): four were infected intrautero and one intrapartum. The higher frequency of intrautero transmission was statistically significant ($P = 0.001$). Use of antiretrovirals (ARVs) in the three stages of gestation was a protective risk factor for MTCT (PR = 0.42; CI: 0.21–0.83; $P = 0.013$). A higher HIV viral load at delivery was the only independent risk factor for MTCT. Early and universal access to ARVs during pregnancy is the most important measure to decrease vertical HIV-1 transmission even in areas where HIV clade distribution differs.

Keywords: South America, women, HIV

INTRODUCTION

The mother-to-child transmission (MTCT) of HIV-1 can occur before, during and after delivery.[1] Transmission can result from microtransfusions, ruptures in the membrane[2] or due to a progressive infection of the placenta's trophoblasts.[3] During delivery, transmission mainly occurs when the child comes in contact with contaminated maternal mucous or fluids while passing through the birth canal or through the neonatal digestive tract.[4] The postnatal transmission occurs primarily when the child is breastfed.[5]

HIV-1 MTCT can be influenced by several factors such as the mode of delivery, the use of antiretroviral (ARV) therapy, prematurity and a high maternal viral load.[6–8] Along with these factors, the viral genetic diversity seems to have an important role in MTCT and in the timing of transmission. Some reports have suggested that subtype C of HIV-1 might be related to a greater proportion of intrauterine transmission[1] when compared with other subtypes. Kwiek *et al.*[9] studied the relationship between MTCT and genetic diversity of transmitted subtype C HIV-1 and observed different diversity patterns during intrauterine and intrapartum transmission. Intrauterine infected-infants tended to be infected with one single variant that was more often detected in the mother's plasma, whereas intrapartum infected-infants showed multiple variants of detected and undetected variants of the mother's quasispecies. Regardless of time of transmission, nearly 50% of the quasispecies included the transmission of variants that were not detected in the mother's blood plasma, suggesting a genetic bottleneck and arguing against a stochastic model of vertical transmission.

The city of Rio Grande, situated at the south shore of the State of Rio Grande do Sul (RS), is home of the greatest portuary complex of the state, which is responsible for an intense flow of people from around the globe. In 27 years of HIV epidemic, Rio Grande presented 1319 diagnosed cases and is one of the 10 most affected cities in RS. A total of 526 women and 58 children (14 years or less) have been infected by the virus between 1980 and 2007.[10] HIV-1 subtype C, responsible for 50% of infections worldwide,[11,] presented a clear expansion in the south of Brazil: from 22% in 2002 to 70% in 2006 in the city of Rio Grande.[12,13] Under this scenario, this study was carried out with the objectives of determining the rate of MTCT, the timing of transmission and the transmitted subtype, associating these findings to demographical, clinical and viral factors in a population where the virus of subtype C prevails.

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PATIENTS AND METHODOLOGY

Study samples

Babies born to HIV-1-infected mothers, followed-up at the University Hospital Dr Miguel Riet Corrêa Jr at Universidade Federal do Rio Grande (FURG) between July 2003 and July 2007, were included in the study. The mothers signed an informed consent to participate in this research and this study was approved by FURG's Ethical Committee.

Molecular analyses

In order to determine the status of the newborn's HIV infection and its moment of transmission, a polymerase chain reaction (PCR) was conducted on proviral DNA extracted from the newborn's first sample (collected at 24/48 hours after birth), at 30 and 60 days of age. Genomic DNA was isolated with the Illustra® Q2 blood genomic Prep Mini Spin (GE Healthcare). A nested PCR was used for amplifying the immuno-dominant region of HIV-1 gp41 genomic region. The primers used in the first round were JH38 (5′ CAGCAGGAAGCACTATGGG 3′) and JH41 (5′ GGTGAGTATCCCTGCCTAAC 3′) and in the second round were Menv27 (5′ AAGCCTCCTACTATCATTATGA 3′) e Env19 (5′ CTGGTATAGTGCAACAGCA 3′).

HIV-1 infection was considered positive if the child presented a positive PCR or two detectable viral loads and negative if he/she presented two negative PCR tests or two undetectable viral loads. Newborns with a positive PCR in the sample collected at 24/48 hours after birth were considered to have intrauterine infection and those who presented a negative PCR at birth and positive in subsequent tests were considered to have intrapartum transmission. HIV viral load was measured by SIEMENS Versant HIV RNA 3.0 assay (bDNA).

Sequencing was performed in an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA). Reactions were carried out according to the manufacturer's recommendations. Sequences were edited in Lasergene software Q3 package (DNAStar Inc, Madison, WI, USA) and then aligned in the Clustal W program. In order to determine the HIV-1 subtype, sequences were aligned with a set of reference HIV-1 subtype sequences obtained from the Los Alamos National Laboratory's HIV Database (http://hiv-web.lanl.gov). A phylogenetic analysis using the neighbour-joining method and the correction of Kimura two-parameters were conducted with all the sequences and the samples had their specific HIV subtype attributed when they grouped to reference sequences of that subtype. The phylogenetic inference was conducted in the MEGA 4.0 program and the phylogenetic tree was drawn in TreeView. The robustness of the different clades on the tree was evaluated by the bootstrapping technique with 2000 replications in the MEGA program.

Statistical analyses

The variables analysed to determine the risk factors for HIV transmission were: mothers' age; race; use of ARV categorized as complete (use of ARV in the three stages of protocol 076: before and during delivery plus azidothymidine (AZT) to the newborn for 6 weeks), incomplete (failure to use ARV in one or more stages) and none; ARV prophylaxis was considered positive if the mother received any ARV during pregnancy; attendance to prenatal care; time of HIV diagnosis in the mother; presence of ruptured membrane at delivery; mode of delivery; CD4+ T-cells count and viral load at delivery (collected during the 34th week of gestation) and viral subtype. Regarding the children, risk factors evaluated were gender, birth weight, timing of transmission and transmitted viral subtype.

Data analysis was performed using Stata version 9.0 statistical software (Stata Corp, College Station, TX, USA). To evaluate the difference in the timing of transmission between intrauterus and delivery transmission, a one sample test for proportions was used. Crude prevalence ratios (PR) and 5% confidence intervals (95% CI) between outcome and each factor were calculated in the univariate analysis. All variables that in the univariate analysis had a P value ≤ 0.20 were included in the multivariate analysis. For this instance a backward stepwise Poisson regression with robust confidence intervals was performed, and adjusted prevalence ratios (aPR) and 95% CI were obtained. Variables with a P value ≤ 0.05 were maintained in the model. In order to study the significance of the associations, a Wald test was used. For all statistical analysis a P value of 0.05 of a two-tailed test was adopted.

RESULTS

Characteristics of the sample

One-hundred and fifty children born to 134 HIV-infected pregnant women were studied: 15 patients had two consecutive pregnancies and one patient had a gemelar gestation. Six children were excluded because of neonatal death, death before 30 days of life or lost to follow-up. A total of 144 children were included in the study. Among the mothers, two cases of seroconversion occurred during pregnancy.

Rate, moment of transmission and transmitted subtype

The rate of vertical transmission among children born within the studied period was of 4.86% (7 of the 144 determined cases); five (71.5%) were men. The subtype of the infected children was determined: five (71.5%) were infected by subtype C of HIV-1 and two (28.6%) by subtype B (Table 1). Five children had their timing of transmission defined: four (80%) were infected intrautero (3 children with subtype C and 1 child with subtype B) and one was infected intrapartum (subtype C). The higher frequency of intrautero transmission was statistically significant ($P = 0.001$). Treatment history for those infected intrautero reveals that only one mother started ARV at 34 week of gestation and three had received AZT only at labour. All children in this group received AZT after birth. Children infected intrapartum received only AZT after birth.

According to the univariate statistical analysis (Table 1), the risk factors for MTCT of HIV were failure to use ARV at the three stages of protocol 076, late diagnosis of HIV status of the mother and a higher viral load at delivery. In the multivariate prediction model (Table 2), with the variables adjusted among themselves, the use of ARV in the three stages of gestation was a protector risk factor for MTCT (PR = 0.42; CI: 0.21–0.83; $P = 0.013$) and a higher HIV viral load at delivery was the only independent risk factor for MTCT: each elevation of a log incremented the risk of vertical transmission by 75% (CI: 1.26–2.45; $P = 0.001$).

DISCUSSION

The global rate of MTCT obtained in this study (4.86%) is considered high when compared with other countries that, like Brazil, have national programmes to reduce vertical transmission that have reached transmission rates lower than 1%.[14] However, it is interesting to analyse our MTCT rates in distinct periods within the same study. Between July 2003 and July 2005 the rate was of 9% and between August 2005 and July 2007, of 1.1%. This important decrease shows the efficiency of the

Table 1 **Risk factors for mother-to-child transmission of HIV in 144 children**

Characteristic	HIV-negative (*n* = 137)	HIV-positive (*n* = 7)	PR*	*P*	95% CI
Mean mother's age	26.3	25.7	0.98	0.805	0.84–1.14
Mother's race					
White (%)	74.8	71.4	0.84	0.841	0.17–4.21
Children's gender					
Male (%)	46	71.5	2.79	0.212	0.55–14.01
Mean birthweight (g)	2948	2725	0.99	0.268	0.99–1.00
>2500 g	85.2	85.7	1.04	0.969	0.13–8.27
Use of ARV† during pregnancy (%)					
None	2.3	42.8	1		
Incompleted	21.4	28.6	0.13	0.001	0.02–0.63
Completed	76.3	28.6	0.03	0.000	0.007–0.19
ARV prophylaxis during pregnancy (%)					
No	14.1	71.4	1		
Yes	85.8	28.5	0.08	0.002	0.01–0.40
Attendance to antenatal care (%)					
Yes	91.2	85.7	0.6	0.625	0.07–4.64
Time of HIV mothers'diagnosis (%)					
Before conception	63.7	42.8	1		
During gestation	28.8	14.3	0.74	0.794	0.07–6.96
At delivery	7.5	42.9	6.84	0.012	1.53–30.56
Ruptured membranes (%)					
At delivery	87.7	85.7	1		
Before delivery	12.3	14.3	1.17	0.877	0.14–9.25
Mode of delivery					
Vaginal (%)	31.6	42.9	1.58	0.538	0.36–6.81
Mean CD4+ T-cell counts (*n*)	540 (130)	418 (6)	0.99	0.296	0.99–1.00
Mean HIV viral load at delivery (log)	1.55 (130)	4.08 (6)	2.16	0.008	1.22–3.82
Maternal HIV subtype (%)					
Non-C	27.6	28.6			
C	72.4	71.4	0.98	0.956	0.60–1.60

*Prevalence ratio
†Antiretroviral

measures used to control MTCT of HIV-1 in Rio Grande. It is also interesting to compare these results to previous works developed in the same city: Martínez *et al.*[13] determined 11.8% of vertical transmission among children born between 1998 and 2003. Similar decreases are seen in several countries that adopted control measures[15,] especially the use of highly active antiretroviral treatment therapy on HIV-positive pregnant patients.

In this study, the proportion of children infected intrautero was greater than to those who were infected during delivery. Magder *et al.*[16] had already demonstrated this tendency and the significant inversion of proportion between intrautero transmissions and those which occur during delivery. It is still uncertain which biological mechanisms are involved in MTCT as the placental expression of receptors and co-receptors for HIV are controversial.[17,18] It was shown that HIV can trespass the barrier in the absence or low expression of receptors/co-receptors in trophoblasts using unusual pathways such as endocytosis.[19,20] Moreover, studies suggest that the transmitted variants may have adaptive, replicative and infective advantages when compared with those which are not transmitted.[18] Some studies suggest that children infected intrautero can progress to AIDS faster,[21,22] although the mortality does not differ between those infected *in uterus* and during delivery.[23] Finally, it is possible that the amniotic fluid does not play an important role in intrauterine MTCT among women who receive ARV.[24,25] However, it was not possible to exclude this hypothesis from this work since the mothers who transmitted the virus intrauterus had not received a complete ARV scheme due to a late start in the antenatal treatment or to adherence failure. Our findings that intrautero transmission was related to a high viral load at delivery is in agreement with other authors,[8,16] but is contrary to the observation made by Mphatswe *et al.*[26] that mothers who transmitted during gestation/pregnancy had a lower viral load when compared with those who transmitted during delivery.

Table 2 **Multivariate analyses of risk factors to HIV mother-to-child transmission**

	Crude PR*			Adusted PR		
Caracteristics	**PR**	**IC**	***P***	**PR**	**IC**	***P***
Child gender	2.79	0.55–14.0	0.212	2.08	0.42–10.3	0.367
Use of antiretroviral during pregnancy	0.19	0.07–0.46	0.000	0.42	0.21–0.83	0.013
Time of HIV mother's diagnosis	2.61	0.94–7.28	0.066	1.22	0.59–2.52	0.585
CD4+ T-cell counts	0.99	0.99–1.00	0.296	0.99	0.99–1.00	0.860
Viral load at delivery (log)	2.16	1.22–3.82	0.008	1.75	1.26–2.45	0.001

*Prevalence ratio

The decrease in MTCT during delivery and consequent increase in intrauterine transmission cases are mainly due to the suppression of peripartum risk factors. However, studies show that the virus' genetic diversity may also be an important factor. Odaibo *et al.*[27] suggest that the MTCT may differ according to subtype and Renjifo *et al.*[1] demonstrated that intrauterine transmissions possibly occur more often with subtype C. In our study, HIV subtype C prevailed among the pregnant women and was, therefore, the predominant subtype transmitted to the children. The low number of patients with other subtypes

did not allow us statistical power to associate the maternal viral subtype with the timing of transmission, but we agree with some authors,[1] who suggest that children infected with subtype C have the tendency of being contaminated in-uterus and may not be protected if their mothers are treated with ARV by the end of pregnancy or at birth.

In this work, we observed only one case (20%) of peripartum transmission of HIV. The mother, despite having received antenatal care, did not adhere completely to the proposed scheme, presented a high viral load at delivery and arrived at the obstetrician already in labour. In developed countries, perinatal transmissions are residual and occur mainly due to seroconversion during pregnancy.[28] Interestingly, in our study, we had two cases of seroconversion during pregnancy which resulted in HIV transmission in one case only. The non-transmitting mother, although unaware of her HIV status, received a high-quality antenatal care, with frequent medical follow-up, which allowed for an adequate clinical health condition.

In this study, the use of ARV in the three stages of pregnancy was a protective factor against MTCT of HIV, while a high viral load at delivery was a risk factor. These results agree with well-established data previously reported.[8,16,29]

In order to initiate prophylaxis at an early stage of gestation and consequently reduce the mother's plasmatic HIV viral load, an early identification of this patient as an HIV-1 carrier is fundamental. Despite having lost its significance in the multivariated analysis, the moment of maternal diagnosis showed an independent importance with respect to the outcome. Studies in developed countries show that routinely testing women during gestation contributes to a great reduction (95%) of MTCT cases. They suggest that the tests should be done at different moments of gestation in order to identify those who became infected during pregnancy.[30] In Brazil, it is recommended that every parturient be tested at delivery if their HIV status is unknown.[31]

The mechanisms through which MTCT occurs are not yet fully clear, and should be a focus point for researchers not only to understand the biology of viral transmission but also in an attempt to eliminate one of the transmission routes. Further studies are also needed regarding the mechanisms that determine the moment of transmission and the influence of the viral subtype. For a population as the one of Rio Grande, located in the south of Brazil, for which Soares *et al.*[32] suggested a frequent recombination between strains and Santos *et al.*[33] described a new CRF possibly due to the intense flow of people, it is pertinent to keep studying the genetic diversity and MTCT. Despite the fact that the rate of MTCT has been decreasing along the studied years, the proportion of intrauterine infections has increased as a result of a successful suppression of the factors which cause peripartum and postnatal transmission. It is still uncertain as to why some children are contaminated and some are not, but it became clear in this study that mothers who did not adhere to treatment or sought early antenatal services to get tested and, if needed, promptly initiate prophylaxis, can transmit the virus to their children. Early and universal access to ARV during pregnancy is the most important measure to achieve a decrease in vertical transmission even in areas where clade distribution differs.

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REFERENCES

1 Renjifo B, Gilbert P, Chaplin B, *et al.* Preferential *in-utero* transmission of HIV-1 subtype C as compared to HIV-1 subtype A or D. *AIDS* 2004;**18**:1629–36
2 Kwiek JJ, Mwapasa V, Milner DA, *et al.* Maternal–fetal microtransfusions and HIV-1 mother to child transmission in Malawi. *PloS Med* 2006;**3**:170–5
3 Vidricaire G, Imbeault M, Tremblay M. Endocytic host cell machinery plays a dominant role in intracellular trafficking of incoming human immunodeficiency virus type 1 in human placental trophoblasts. *J Virol* 2004;**78**:11904–15
4 Nielsen K, Boyer P, Dillon M, *et al.* Presence of immunodeficiency virus (HIV) type 1 and HIV- specific antibodies in cervicovaginal secretions of infected mothers and in the gastric aspirates of their infants. *J Infect Dis* 1996;**173**:1001–4
5 Embree JE, Njenga S, Datta P, *et al.* Risk factors for postnatal mother to child transmission of HIV-1. *AIDS* 2000;**14**:2535–41
6 Jamieson DJ, Read JS, Kourtis AP, Durant TM, Lampe MA, Dominguez KL. Cesarean delivery for HIV-infected women: recommendations and controversies. *Am J Obstet Gynecol* 2007;(Suppl September):S96–100 Q4
7 Zijenah LS, Moulton LH, Iliff P, *et al.* Timing of mother to child transmission of HIV-1 and infant mortality in the first 6 months of life in Harare, Zimbabwe. *AIDS* 2004;**18**:273–80
8 Jourdain G, Mary JY, Le Coeur S, *et al.* Risk factors for *in utero* or intrapartum mother-to-child transmission of human immunodeficiency virus type 1 in Thailand. *J Infect Dis* 2007;**196**:1629
9 Kwiek JJ, Russell ES, Dang KK, *et al.* The molecular epidemiology of HIV-1 envelope diversity during HIV-1 subtype C vertical transmission in Malawian mother–infant pairs. *AIDS* 2008;**22**:863–71
10 Ministério da Saúde. *Datasus: Informações de Saúde*. See [http://www.aids.gov.br/cgi/deftohtm.exe?tabnet/br.def] (last checked 20 April 2008)
11 Buonaguro L, Tornesello ML, Buonaguro FM. Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. *J Virol* 2007;**81**:10209–19
12 de Martínez AM, Barbosa EF, Ferreira PC, *et al.* Molecular epidemiology of HIV-1 in Rio Grande, RS, Brazil. *Rev Soc Bras Med Trop* 2002;**35**:471–6
13 Martínez AM, Hora VP, Santos AL, *et al.* Determinants of HIV-1 mother-to-child transmission in Southern Brazil. *An Acad Bras Cienc* 2006;**78**:113–21
14 Avettand-Fenoel V, Chaix ML, Blanche S, Burgard M, Warszawski J, Rouzioux C. Early diagnosis of HIV-1 infection in newborns, in the context of prevention of mother-to-child transmission with HAART (Perinatal Cohort ANRS Co 01). *Retrovirology* 2008;**5**(Suppl. 1):O24
15 Plipat T, Naiwatanakul T, Rattanasuporn N, *et al.* Reduction in mother-to-child transmission of HIV in Thailand, 2001–2003: results from population-based surveillance in six provinces. *AIDS* 2007;**21**:145–51
16 Magder LS, Mofenson L, Paul ME, *et al.* Risk factors for *in utero* and intrapartum transmission of HIV. *J Acquir Immune Defic Syndr* 2005;**38**:87–95
17 Bustamante S, Garcia Y, Garrido H, *et al.* CXCR-4 AND CCR-5 expression in normal term human placenta. *Invest Clin* 2005;**46**:25–35
18 Margolis L, Shattock R. Selective transmission of CCR5-utilizing HIV-1: the 'gatekeeper' problem resolved ? *Nat Rev Microbiol* 2006;**4**:312–17
19 Vidricaire G, Gauthier S, Tremblay M. HIV-1 infection of trophoblasts is independent of gp120/CD4 interactions but relies on heparan sulfate proteoglycans. *J Infect Dis* 2007;**195**:1461–71
20 Tremblay MJ, Vidricaire G. HIV-1 internalization in polarized human trophoblasts occurs through a peculiar endocytic pathway. *Retrovirology* 2008;**5**(Suppl. 1):5
21 Fawzi W, Msamanga G, Renjifo B, *et al.* Predictors of intrauterine and intrapartum transmission of HIV-1 among Tanzanian women. *AIDS* 2001;**15**:1157–65
22 Mock PA, Shaffer N, Bhadrakon C, *et al.* Maternal viral load and timing of mother-to-child HIV transmission, Bangkok, Thailand. *AIDS* 1999;**13**:407–14
23 Fox MP, Brooks D, Kuhn L, *et al.* Reduced mortality associated with breast-feeding-acquired HIV infection and breast-feeding among HIV-infected children in Zambia. *J Acquir Immune Defic Syndr* 2008;**48**:90–6

24 Maiques V, García-Tejedor A, Perales A, Córdoba J, Esteban RJ. HIV detection in amniotic fluid samples. Amniocentesis can be performed in HIV pregnant women? *Eur J Obstet Gynecol Reprod Biol* 2003;**108**:137–41
25 Mohlala BKF, Tucker TJ, Besser MJ, *et al.* Investigation of HIV in amniotic fluid from HIV-infected pregnant women at full term. *J Infect Dis* 2005;**192**:488–91
26 Mphatswe W, Blanckenberg N, Tudor-Williams G, *et al.* High frequency of rapid immunological progression in African infants infected in the era of perinatal HIV prophylaxis. *AIDS* 2007;**21**:1253–61
27 Odaibo GN, Olaleye DO, Heyndrickx L, Vereecken K, Houwer K, Jassens W. Mother-to-child transmission of different HIV-1 subtypes among ARV naïve infected pregnant women in Nigeria. *Rev Inst Med Trop Sao Paulo* 2006;**48**:77–80
28 Moses SE, Tosswill J, Sudhanva M, Poulton M, Zuckerman M. HIV-1 seroconversion during pregnancy resulting in vertical transmission. *J Clin Virol* 2008;**41**:152–3
29 Newell ML. Mechanisms and timing of mother-to-child transmission of HIV-1. *AIDS* 1998;**12**:831–7
30 Patterson KB, Leone PA, Fiscus SA, *et al.* Frequent detection of acute HIV infection in pregnant women. *AIDS* 2007;**21**:2303–8
31 Ministério da Saúde. Recomendações para profilaxia da transmissão vertical do HIV e terapia anti-retroviral em gestantes. *Série Manuais* 2004;**46**:49–50
32 Soares MA, Oliveira T, Brindeiro RM, *et al.* A specific subtype C of human immunodeficiency virus type 1 circulates in Brazil. *AIDS* 2003;**17**:11–21
33 Santos AF, Sousa TM, Soares EAJM, *et al.* Characterization of a new circulating recombinant form comprising HIV-1 subtypes C and B in southern Brazil. *AIDS* 2006;**20**:2011–9

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