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HIV-1 mother-to-child transmission and drug resistance among Brazilian pregnant women with high access to diagnosis and prophylactic measures

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ABSTRACT

Background: A high-coverage public health prenatal program (70,000 women/year) from central western Brazil/Goias State has represented a unique opportunity for the early diagnosis of HIV-1 and implementation of strategies to prevent mother-to-child transmission (MTCT).
Objectives: To investigate MTCT among a prospective cohort of HIV-1 infected mothers/exposed infants.
Study design: 142 mothers/their 149 infants (2008–2010) were investigated regarding maternal viral load, CD4$^+$cell counts, HIV-1 *pol* sequences; infants' HIV-1 RNA tests (30/120days), sequential anti-HIV-1/2 serology. HIV-1 subtypes were assigned by REGA. Transmitted drug resistance was identified by the Calibrated Population Resistance tool, secondary resistance by Stanford HIV-1 Drug Resistance/International AIDS Society databases.
Results: Mothers (median age = 24 years; 25/142 adolescents) were diagnosed during prenatal care (2008–2010) or previously (1994–2007). Recent cases were younger, mostly asymptomatic. Undetectable viremia and MTCT prophylaxis predominated in formerly diagnosed mothers. Recent cases had higher subtype C prevalence. One naive patient had transmitted resistance; ten antiretroviral-experienced patients had secondary resistance: 6 from MTCT prophylaxis, 4 under HAART. Late disclosure of diagnosis, vaginal delivery, breastfeeding, lack of oral zidovudine were observed in the three MTCT cases (3/149; 2.01%). Two of three infected infants harbored subtype C; infected infants/mothers did not have drug resistance mutations. Two of the transmitting-mothers had viremia <1000 copies/ml. Among exposed-uninfected infants the median time to seroreversion was 12 months.
Conclusions: In this study delayed disclosure of diagnosis, partial/no preventive measures, drug resistance among asymptomatic women under prophylaxis and MTCT in low viremic mothers raise concerns. The expansion of subtype C infection corroborates surveillance of HIV-1 diversity in this region.

1. Background

HIV-1 mother-to-child-transmission (MTCT) is a multifactorial event in which high maternal viral load, low CD4$^+$ cell counts, mode of delivery, antiretroviral (ARV) therapy and prematurity play a role.[1] Preventive strategies including prophylactic or therapeutic ARV use, cesarian section and breastfeeding proscription can reduce MTCT from 25% up to 1%.[2,3] However, ARV treatment can promote the selection and transmission of resistant mutants and compromise prevention.[4,5]

Brazil was the first developing country to implement a countrywide public health program to prevent HIV-1 MTCT, however it still occurs.[6,7] Routine prenatal HIV-1 screening represents a unique circumstance for the early diagnosis and prevention of MTCT. Nevertheless, missing opportunities can jeopardize preventive measures.[8]

In Goias State, central western Brazil, a special public health prenatal program ("Program for the Protection of Pregnant Women/PPPW") implemented in 2003 screens ~70,000 pregnant women/year from ~240 municipalities. Serological screening includes HIV-1, hepatitis B/C, Human T-Lymphotropic-Virus, syphilis, toxoplasmosis, rubella, Chagas' disease and

Abbreviations: HIV-1, human immunodeficiency virus type 1; MTCT, mother-to-child transmission; ARV, antiretroviral; PPPW, Program for the Protection of Pregnant Women; HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse-transcriptase inhibitors; NNRTI, nonnucleoside reverse-transcriptase; PI, inhibitor protease; PR, protease; RT, reverse transcriptase; cDNA, complementary DNA; CPR, Calibrated Population Resistance Tool; IAS-USA, International AIDS Society-USA; CI, confidence Interval; AZT, zidovudine; MDR, multidrugresistant.

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Cytomegalovirus. A survey among 28,561 pregnant women from this regional Program showed an HIV-1 prevalence of 0.09% (95% CI 0.06%–0.14%).[9] A recent report by the Ministry of Health showed important regional differences in the rate of HIV-1 MTCT in Brazil: highest rate in southern (5.8%) and lowest rate (1%) in central western region.[7]

2. Objectives

This prospective study among pairs of HIV-1 infected mothers and exposed infants from central western Brazil describes MTCT and related factors (prophylaxis, resistance mutations). HIV-1 subtypes and seroreversion in exposed-uninfected children are also reported.

3. Study design

3.1. Patients

During the study period (June/2008–June/2010), 146,897 pregnant women were screened by the PPPW/"Institute of Diagnoses and Prevention/IDP/APAE", Goiania/Goias). Around 70% (148/198) of confirmed cases of HIV-1 infection were enrolled. This cohort included both recently and formerly diagnosed women as prenatal screening includes all pregnant women, regardless of previous HIV-1 diagnosis. Enrollment occurred any time during pregnancy or up to 30 days postpartum. Mother–infant pairs were prospectively followed up (one year) at the main regional public hospital for HIV-1 care (Anuar Auad Hospital, HAA/HDT/SUS). Epidemiological data were collected in standardized forms; clinical, obstetric data were obtained from medical records. This research protocol was approved by the institutional review board ("Comite de Etica e Pesquisa HAA/HDT/SUS", protocol #003/2008). All women provided informed consent for themselves and their newborns.

The standard highly active-antiretroviral-therapy/HAART regimen used for MTCT prophylaxis/treatment consisted of two NRTIs: zidovudine (ZDV)/3TC–Combivir® and one PI (LPV/ritonavir-boosted–Kaletra®).[10]

3.2. Immunological and virological profiles

The classification into asymptomatic/symptomatic was defined at diagnosis according to CDC AIDS-defining conditions. $CD4^+$ cell counts (FACSCalibur, Becton & Dickson, San Jose, CA, USA), HIV-1 RNA tests (Amplicor HIV-1 Monitor test, version 1.5; Roche, USA) were performed in maternal samples (last trimester-30 days postpartum) and in infants (30, 120 days of life). MTCT diagnosis was based on two positive HIV-1 RNA tests in samples collected after 30 days of life. Two negative HIV-1 RNA tests (30/120 days of life) excluded MTCT. Exposed-uninfected newborns were monitored by anti-HIV-1/2 IgG ELISA (Wiener Laboratories, Argentina) each 3–4 months throughout the first 12 months or until seroreversion.

3.3. HIV-1 subtypes and resistance analysis in pol gene

Plasma RNA (mothers/infected newborns) was extracted, retrotranscribed into complementary DNA (cDNA) and the entire HIV-1 protease (PR) and reverse transcriptase (RT) fragment (~750-bp) were amplified by nested polymerase chain reaction followed by direct sequencing, as described.[11] HIV-1 subtypes were identified using REGA tool version 2.0.[12]

Transmitted drug resistance was identified by the Calibrated Population Resistance tool[13] secondary drug resistance by the Stanford Surveillance Drug Resistance Mutation/International AIDS Society-USA (IAS-USA) major mutation list (Accessed: August/2011). GenBank accession numbers of the generated sequences are JN114115, JN114116,

Table 1
Main epidemiological, clinical and MTCT prophylaxis characteristics among newly diagnosed mothers for HIV-1 infection and mothers diagnosed before index pregnancy.

Variable	Recently diagnosed mothers ($n=65$)	Formerly diagnosed mothers ($n=77$)	P
Age at diagnosis (years)			
Median (range)	25 (15–39)	23 (14–36)	0.04
Clinical status at diagnosis n/N (%)			
Asymptomatic (HIV)	56/62[a] (90.3.0)	54/76[b] (71.1)	0.04
Symptomatic (AIDS)	06/62 (9.7)	22/76 (28.9)	
$CD4^+$ cell counts (cells/mm^3)[A]			
Median (range)	543 (162-1418)[c]	499 (91–1931)[d]	0.21
Plasma viral load[A] **(copies/mL)**			
Median (n)	19,826 (35)	5836 (27)	0.007
Range	492–750,000	455- 312,349	
Undetectable n (%)	22/57[e] (38.6)	43/70[f] (61.4)	0.01
ARV during pregnancy[A] **n (%)**			
1st or 2nd trimester	38 (58.5)	56 (72.7)	0.04
3rd trimester	14 (21.5)	08 (10.4)	
No ARV	13 (20.0)	13 (16.9)	
Maternal IV ZDV during labor[A] **n/N (%)**			
Yes	56/62[g] (90.3)	72/75[h] (96.0)	0.16
No	06/62 (9.7)	03/75 (4.0)	
Mode of delivery[A]			
Cesarian section n/N (%)	43/65 (66.1)	63/77 (81.8)	0.05
Vaginal n/N (%)	22/65 (33.9)	14/77 (18.2)	
HIV-1 Subtypes in pol[B] **n/N (%)**			
B	25/40 (62.5)	29/43 (67.5)	0.40
BF1	04/40 (10.0)	09/43 (20.9)	0.14
C	09/40 (22.5)	02/43 (4.6)	0.02
F1	02/40 (5.0)	02/43 (4.6)	0.66
CB	00 (0.0)	01/43 (2.3)	0.51

The P values were determined by Fisher's exact test, Mann–Whitney or Spearman test, as appropriate [CI 95% ($p<0.05$)]; ARV = Antiretroviral; ZDV = zidovudine; Missing data: $a=03$, $b=01$; $c=08$; $d=06$; $e=08$; $f=07$; $g=03$; $h=2$.

[A] Spearman tests considering: ARV therapy as covariate to compare $CD4^+$ T cell counts and viral loads for the two groups ($r=0.11$; $r=0.24$, respectively); time of diagnosis as a covariate to compare prophylaxis, maternal intravenous ZDV use during labor and mode of delivery ($r=-0.25$; $r=0.22$; $r=0.16$, respectively).

[B] Not amplified = 59.

JN114118, JN114120, JN114124, JN114126, JN114127, JN114129, JN114130, JN114132–JN114138, JN114140–JN114145, JN114147, JN114149–JN114153, JN114155, JN114159, JN114162, JN114164, JN114168, JN1141, JN114170, JN114171, JN114173–JN114176, JN114178–JN114180, JN114182–JN114188, JN114190–JN114195, JN114199, JN114201, JN1142, JN114203, JN114206, JN114207, JN114209, JN114210, JN114217, JN114222, JN114225, JN114227, JN114229, JN114235; JN662426, JN662427, JN662429, JN662431, JN662434, JN662435.

3.4. Statistical analyses

Descriptive analyses (frequency, medians, averages, standard deviations) (Epi Info™ Version 3.5.1) and univariate analyses were performed. Fisher Exact, Spearman, Kruskal Wallis or Mann Whitney tests (95%CI, p-value < 0.05) were performed when appropriate.

4. Results

4.1. Characteristics of pregnant women infected with HIV-1

Among 148 pairs of HIV-1 infected mothers/exposed infants studied, six women were enrolled twice due to consecutive pregnancies; one mother gave birth to twins. Therefore this cohort consisted of 142 different mothers that delivered a total of 149 live born infants. No miscarriage or stillbirth was reported.

The median maternal age at diagnosis was 24 years (range 14–39 years; sd = 5.3), adolescents (14–19 years old) represented 17.6% (25/142). Heterosexual transmission was reported by all participants; however 10.6% (15/142) referred blood transfusion, 2.1% (3/142) were intravenous drug users. Mothers had low educational level/income: 4.9% (7/142) were illiterate, 45.8% (65/142) had <8 years of education, 23.9% (34/142) had a formal job with salary.

For the six mothers with consecutive pregnancies, data for both pregnancies were collected; Table 1 data refer to the first pregnancy. Comparison of data from consecutive pregnancies indicated lower $CD4^+$ cell counts (5/6 women) and higher viral loads (3/6 women) in the second pregnancy. Among the 12 babies born to these six mothers, one case of MTCT was diagnosed in the first pregnancy of one of them.

The great majority of participants (83.8%; 119/142) were diagnosed for HIV-1 during prenatal care: 65 from 2008 to 2010 at a median gestational age of 20 weeks (recently diagnosed group) and 54 were originally diagnosed from 1994 to 2007 (formerly diagnosed group) (median time since diagnosis = 4 years; 1–14 years range). Among formerly diagnosed group only 16.2% (23/142) were diagnosed for HIV-1 infection independently of a prenatal care. Among recent cases, three women were diagnosed during labor (HIV1/2 rapid tests); for other three participants HIV-1 diagnosis was disclosed 10 days postpartum.

Comparisons between recently/formerly diagnosed women (Table 1) indicate mostly asymptomatic women at the time of diagnosis. However higher rate of symptomatic cases was observed among formerly diagnosed (28.9% *vs* 9.7%; $p < 0.05$), the majority (49/77; 63.6%) was under ARV when they became pregnant, most (72.7% *vs* 58.5%) received early MTCT prophylaxis ($p < 0.05$), which correlated with higher rate of undetectable viremia (61.4% *vs* 38.6%) ($p < 0.05$). High access to intravenous ZDV during labor (>90%) and cesarian section predominated in both groups. Among adolescent pregnant sub-group ($n = 25$), most (14/25) were multiparous; 12/25 were diagnosed for HIV-1 during a previous prenatal care. Around 25% (6/25) of adolescents were symptomatic. Most adolescents initiated early prophylaxis and all received ARV-prophylaxis/treatment. No MTCT case was observed among adolescents.

Table 2
Amino acid substitutions in the PR (protease) and RT (reverse transcriptase) resistance-related codons of mothers with secondary resistance to antiretroviral drugs.

PR/RT	Therapy/prophylaxis	Subtype	Protease inhibitors (PI)		Reverse transcriptase inhibitor mutations		Resistance profile		
			Major mutations	Minor mutations	NRTI	NNRTI	Low	Intermediate	High
JN114124	HAART therapy	B/B	–	–	M184V	–	–	–	3TC/FTC
JN114124	HAART therapy	F1/B	–	–	A62V, M184V	K103 N, P225H			3TC/FTC e DLV/EFV/NVP
JN114132	HAART therapy	F1/B	–	–	M184V, T215Y	K103 N, P225H	ETR ZDV/D4 T/DDI	– ABC	3TC/FTC e DLV/EFV/NVP
JN114143	HAART therapy	B/B	D30N, N88D, L90M	A71 T	D67N, K70R, M184V, T215Y, K219DE	L100I, H221Y	FPVr TDF ETR	ATVr/IDVr/SQVr ABC/D4 T/DDI DLV/EFV/NVP	NFV 3TC/AZT/FTC
JN114118	HAART prophylaxis[a]	B/B	M46L, I50L, V82A	A71V	M184V	–	FPVr/SQVr	IDVr/LPVr	ATVr/ NFV
JN114145	HAART prophylaxis[a]	C/C	–	–	M184V	K103 N	–	–	3TC/FTC DLV/EFV/NVP
JN114173	HAART prophylaxis	B/B	I85V	–	–	–	–	–	–
JN114190	HAART prophylaxis	F1/B	–	–	A62V, T69A, M184M	–	ABC	–	3TC/FTC
JN114207	HAART prophylaxis	B/B	–	–	–	V106I, V179D, Y188L	ETR	DLV	EFV/NVP
JN114227	HAART prophylaxis[a]	B/F1	–	–	M184V	E138A	–	–	3TC/FTC

PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors. ATV: atazanavir; ZDV: zidovudine; DDI: didanosine; DLV: delavirdine; d4T: stavudine; EFV: efavirenz; ETR: etravirine; FPV: fosamprenavir; IDV: indinavir; LPV: lopinavir; NFV: nelfinavir; NVP: nevirapine, SQV: saquinavir.
[a] Patient with former diagnosis which received MTCT prophylaxis in a previous pregnancy.

4.2. HIV-1 pol gene diversity, transmitted and secondary drug resistance

Pol was sequenced in 58.4% (83/142) participants, all of them from central western Brazil (80 from Goias/3 from Mato Grosso State). Forty patients were recent diagnoses, 43 were formerly diagnosed (Table 1). Interestingly HIV-1 subtype C prevalence was higher among recent cases (22.5% *vs* 4.6%) ($p < 0.05$). Overall HIV-1 subtype B predominated, followed by BF1 recombinants; similar frequency of subtype F1 (~5%) was observed in both groups. HIV-1 subtype B ($n = 2$) and one BF1 isolate were detected in patients from Mato Grosso.

Only 1 out of 17 ARV-naive patients presented transmitted resistance: M46L PI mutation. In the ARV-experienced group, (57 under prophylaxis; 9 under HAART), ten patients (15.2%) presented secondary drug resistance: six under prophylaxis, four under HAART (Table 2). Single class resistance mutations were observed in three recent cases under prophylaxis: PI (JN114173), NRTI (JN114190) and NNRTI (JN114207). In the prophylaxis group, 3 formerly diagnosed patients submitted to previous prophylaxis presented dual class mutations: PI/NRTI (JN114118) and NRTI/NNRTI (JN114145, JN114227). In the HAART group NRTI mutation (JN114124), NRTI/NNRTI mutations (JN114124, JN114132) and multidrug resistance (MDR) (JN114143) were detected.

4.3. Main characteristics of HIV-1 exposed infants

Most HIV-1 exposed infants were males, <10% were premature (<37 weeks) (Table 3). Around 6% (6/95) infants born to recently diagnosed mothers were breastfed (~10 days) and missed oral ZDV.

In this cohort the rate of HIV-1 MTCT was 2.01% (3/149) and associated with late disclosure of maternal diagnosis that resulted in two cases of vaginal delivery, one breastfed infant and one case did not take oral ZDV. Among mother/infant pairs that missed opportunities to fully prevent MTCT, no clinical/laboratory differences were observed among the ones that did not transmit ($n = 48$) and the MTCT cases ($n = 3$) (data not shown). Interestingly two out of three MTCT cases harbored HIV-1 subtype C; one was subtype B ($p < 0.05$). None of the transmitting mothers/infected infants had drug resistance mutations in *pol*.

Almost half (69/146) of HIV-1 exposed-uninfected infants were followed up until seroreversion that took place at a median age of 12 months (1–18 months range). Maternal viremia, clinical status, birth weight, gestational age had no impact on the time to

Table 3
Main characteristics of HIV-1 infected mothers/exposed infants ($n = 149$) and of three cases of HIV-1 MTCT.

	All *n* (%)	CASE 1	CASE 2	CASE 3
Infant features	149 (100)			
Gender		F	F	F
F	73 (49)			
M	76 (51)			
Gestational age (weeks)		38	36	38
Median (range)	38 (33–42)			
Birth weight (g)		3095	2600	2430
Median	2970			
(range)	(1548–4710)			
Mode of delivery		Cesarian section	Vaginal	Vaginal
Cesarian	110 (73.8)			
Vaginal	39 (26.2)			
Oral ZDV		Yes	No	Yes
Yes	143 (96.0)			
No	06 (4.0)			
Breastfeeding		No	Yes	No
Yes	06 (4.0)		(10 days)	
No	143 (96.0)			
CD4 T cell counts (cell/mm^3)		2175	NA	937
Median	2812			
(range)	(855–6500)			
Age at HIV-1 diagnosis (days)	NA	30	10	30
Viral load at diagnosis (copies/mL)	NA	34,499	121,468	127,069
Seroreversion (months)[A]		No	No	No
Median (range)	12 (1–18)			
Maternal features	142 (100)			
Clinical status[a]		AIDS	HIV	AIDS
Asymptomatic (HIV)	86 (62.3)			
Symptomatic (AIDS)	52 (37.7)			
CD4 T cell counts[b] (cell/mm^3)		932	NA	303
Median	525			
(range)	(91–1931)			
Plasma viral load[c] (copies/mL)		862	2840	Undetectable
Median	10,209			
(range)	(455–750,000)			
Undetectable	64			
ARV during pregnancy		3rd trimester	No ARV	3rd trimester
1st or 2nd trimester	94 (66.2)			
3rd trimester	22 (15.5)			
No ARV	26 (18.3)			
ZDV in labor[d]		Yes	No	Yes
Yes	128 (93.4)			
No	09 (6.6)			

ARV: antiretroviral; F = female; ZDV: zidovudine; IV: intravenous; NA: not available; missing data: $a = 4$; $b = 14$; $c = 15$; $d = 5$.
[A] 69 children followed up until seroreversion.

seroreversion. Seventy-seven HIV-1 exposed-uninfected children did not comply with extended serological follow up after the early exclusion of MTCT by molecular tests.

5. Discussion

This prospective cohort study discloses important features of HIV-1 infected pregnant women and exposed infants from inland Goias State/central western Brazil, a setting that offers high prenatal access to diagnosis, treatment and MTCT prophylaxis. In summary, the profiles of pregnant women depicted here reflect the current hallmark features of the HIV-1 epidemic in Brazil: "interiorization, heterosexualization, juvenilization and impoverishment".

In our study, around 10% isolates from pregnant women under prophylaxis or continuous HAART had drug resistance mutations. These results raise concerns regarding the efficacy of future MTCT prophylaxis/therapy for these patients. In our study the most prevalent NRTI M184V mutation, leads to resistance to 3TC and increases susceptibility to ZDV/d4T.[14,15] Two women under HAART had the NRTI T215Y mutation that removes ZDV and d4T incorporated into the DNA when associated with M184 V mutation. One MDR patient under HAART had thymidine analogue resistance mutations (TAMs) in codons 67/70/215 and accumulation of TAMs may lead to cross-resistance between NRTIs.[16] In this study none of the HIV-1 transmitting mothers presented resistance mutations. However since our analyses were performed by bulk sequencing, which detects viral populations representing >20%, drug resistance mutations in minority viral populations cannot be excluded.[17]

The rate of HIV-1 MTCT observed here (~2%) is lower than described in other Brazilian studies, which also reported partial access to prophylactic measures.[18–23] In fact recent data showed that central western region has the lowest rate of HIV-1 MTCT in Brazil.[7] These results undoubtedly reflect the positive impact of the wide and efficient public health prenatal "Program for the Protection of Pregnant Women", implemented by two out three States from this region (Goias and Mato Grosso do Sul States).

High viremia during pregnancy/delivery is considered the main risk factor for MTCT.[24–27] However in our study, MTCT occurred in the context of low maternal viremia. Despite the high prenatal coverage/HIV-1 screening in the study area, the MTCT cases observed seemed to correspond to "missing opportunities" to fully prevent transmission.[28,29] A previous regional study showed 40.8% *vs* 1% MTCT without and under adequate prophylaxis.[29] In our study, time of diagnosis also seemed a key event to MTCT outcome, emphasizing the importance of early prenatal care for the diagnosis and treatment/prophylaxis. Early ARV-intervention is crucial to reach undetectable viremia at delivery, which represents one of the cornerstones in MTCT prevention.

The small number of infected children in this study is an acknowledged limitation, indicating that multicentric cohort studies are necessary to investigate risk factors associated with HIV-1 vertical transmission. Since most participants were either under HAART/prophylaxis and therefore had low/undetectable viremia, around 60% had cDNA amplified and HIV-1 *pol* sequenced. Despite this, it is noteworthy that higher prevalence of subtype C was observed among recent cases (13.2%) compared to formerly diagnosed mothers and previous regional surveys (~2.0%).[18,30–32] The current study presents the highest prevalence of subtype C detected in central western Brazil so far. It is also remarkable that two (18.2%) of the 11 mothers infected with subtype C transmitted the virus, while only one of the 72 mothers (1.4%) with "non-subtype C" transmitted HIV-1. While the role of different HIV-1 subtypes in vertical transmission is still debatable[33–35] our study confirms the interiorization of HIV-1 subtype C in inland Brazil among heterosexual population.

The data of HIV-1 infected pregnant women/newborns from inland Brazil described here may help define timely strategies for earlier diagnosis/prophylaxis during prenatal care which are fundamental to progress towards an HIV-free generation. The dissemination of HIV-1 subtype C in an area where subtype B predominates, emphasizes the need of continued molecular surveillance studies in central western Brazil.

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Competing interests

All authors had no conflicts.

Ethical approval

This research protocol was approved by the institutional review board ("Comite de Etica e Pesquisa HAA/HDT/SUS", protocol #003/2008) and all women provided informed consent for enrollment of themselves and their newborns.

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