Breastfeeding, genetic, obstetric and other risk factors associated with mother-to-child transmission of HIV-1 in Sao Paulo State, Brazil

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Objectives: To evaluate the effect of maternal, obstetric, neonatal and post-natal factors on the risk of vertical transmission of HIV-1.

Design: Multicentre retrospective cohort study.

Setting: Obstetric and paediatric clinics in four cities in Sao Paulo State, Brazil.

Main outcome: Child's HIV-1 infection status.

Methods: Data were collected by standardized record abstraction and interview on 553 children born to women identified as HIV-1-infected before or at delivery. Paediatric infection was determined by immunoglobulin G anti-HIV-1 tests at age 18 months or by AIDS diagnosis at any age. Multivariate logistic regression was used to assess the effect of potential risk factors on vertical transmission of HIV-1.

Results: HIV-1 infection status was determined for 434 children (follow-up rate of 78%); 69 were classified as HIV-1-infected [transmission risk, 16%; 95% confidence interval (CI), 13–20%]. In multivariate analysis, advanced maternal HIV-1 disease [odds ratio (OR), 4.5; 95% CI, 2.1–9.5], ever breastfed (OR, 2.2; 95% CI, 1.2–4.2), child's negative Rhesus blood group (OR, 2.5; 95% CI, 1.2–5.5), third trimester amniocentesis (OR, 4.1; 95% CI, 1.2–13.5) and black racial group (OR, 0.3; 95% CI, 0.1–0.9) were independently and significantly associated with mother-to-child transmission of HIV-1. Transmission was increased marginally with prematurity, more than 10 lifetime sexual partners and prolonged duration of membrane rupture. No association was found between child's HIV-1 infection and mode of delivery or serological evidence of syphilis during pregnancy.

Conclusion: These findings support the importance of severity of maternal HIV-1 disease in the risk of vertical transmission of HIV-1, indicate measures to reduce transmission by avoiding amniocentesis and breastfeeding and suggest that race and Rhesus blood type may be markers for genetic susceptibility to infection.

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Introduction

Vertical transmission of HIV-1 is a multifactorial process, and prevalence of factors associated with HIV-1 transmission varies across populations. Studies in Europe, North America and Africa have reported vertical transmission rates between 15 and 35% [1,2] and have identified factors associated with increased risk, such as advanced maternal HIV-1 disease, prolonged duration of membrane rupture (DMR) and breastfeeding. The effects of mode of delivery, invasive procedures during pregnancy and presence of other sexually transmitted diseases have been less consistently quantified [1–8].

Although initiation of zidovudine therapy during pregnancy and its continuation during labour, delivery and the neonatal period has been proven to reduce vertical transmission of HIV-1, it does not eliminate transmission [9–11]. A better understanding is needed of the factors that affect transmission to improve the management of HIV-1-infected women during their pregnancies and to develop alternative methods to reduce vertical transmission of HIV-1. We report here the results of the first large study relating to mother-to-child transmission of HIV-1 in Sao Paulo State, Brazil.

Methods

Study population and data collection

Laboratory records of anti-HIV-1 antibody test results were reviewed in seven maternity hospitals in four cities (Ribeirao Preto, Campinas, Santos and Sao Paulo City) in Sao Paulo State. All women who tested positive before or at delivery and who gave birth to live infants between January 1988 and April 1993 were eligible for inclusion. A woman was considered to be anti-HIV-1-positive if at least two different commercially available enzyme-linked immunosorbent assays (ELISA) were positive on a single maternal sample or if two ELISA were positive on two different samples from the mother or one each from the mother and her newborn [12]. Women were excluded from the study if their child had died during the first 28 days of life (n = 19; causes of neonatal death included obstetric trauma, aspiration, cardiac malformation, necrotizing enteritis and sudden death), if they were living outside the study cities for the last 6 months of pregnancy (n = 72), if a home address was not available (n = 11), or if they had received antiretroviral treatment during pregnancy (n = 2).

Data were collected between October 1993 and April 1995. Information on maternal, obstetric, neonatal and post-natal factors was abstracted from medical records and an interview was conducted after informed written consent had been obtained. Information on AIDS diagnosis and death was sought from the AIDS Registry for the mothers and children who were not found. When possible, and only after checking about the interviewee's awareness of the maternal and infant's diagnosis of HIV-1 infection, interviews were conducted with a family member for women who had died or moved. Ethical approval was obtained from all participating institutions.

Outcome definition and laboratory methods

The child's HIV-1 infection status was determined by immunoglobulin (Ig) G anti-HIV-1 antibody tests in serum or oral fluid at 18 months or later, and by clinical criteria. A child was considered HIV-1-infected if anti-HIV-1 antibody was positive in serum or saliva at or after 18 months of age or if an AIDS-defining illness was diagnosed [13], or at least two major and two minor HIV-1-related signs were present in the absence of known causes of immunosuppression at any age [14,15]. A child was considered not infected if found to be HIV-1 antibody-negative in serum or saliva at or after 18 months of age without clinical signs of AIDS. A child was classified as HIV-1-indeterminate if found to be seropositive when last seen or had died from a non-HIV-1-related disease before 18 months of age [15].

Antibody status was abstracted from the medical records of children who were followed up in the paediatric centres. Sera were collected for the study from children who had not had a serological test performed at or after 18 months of age. Serological tests for detection of anti-HIV-1 and HIV-2 antibodies were performed in duplicate by second generation commercial ELISA according to the manufacturer's instructions. Repeatedly reactive sera were further tested by the Cambridge Biotech HIV-1 immunoblot kit (Cambridge BioScience Corporation, Worcester, Massachusetts, USA). Samples that showed reactivity to any two of the gp160, gp120, gp41, or p24 bands according to Centers for Disease Control and Prevention criteria were classified as HIV-1 antibody-positive [16].

Oral fluid samples were collected using sponge foam swabs and were tested for antibodies to HIV-1 and HIV-2 using an IgG antibody capture ELISA (GACELISA). Samples that were reactive in at least two out of three replicate tests were considered anti-HIV-1 IgG-positive and were confirmed using a modified Western blot procedure (Diagnostic Biotechnology HIV blot 2.2, Genelabs Diagnostics SA, Geneva, Switzerland). When validated against serological antibody testing, salivary testing for HIV-1 antibodies in children demonstrated 100% specificity and sensitivity [95% confidence interval (CI), 99–100 and 90–100, respectively] [17].

Statistical analysis

Potential risk factors were categorized into two or more strata; continuous variables were categorized into quartiles or according to thresholds established *a priori*. Unconditional logistic regression modelling was used to investigate the independent effects of each selected risk factor simultaneously. Risk factors were included in the model if they were significant at $P = 0.10$ or if the crude odds ratio (OR) was ≥ 3.0 or ≤ 0.3 in univariate analysis. The individual contribution of each risk factor was assessed by likelihood ratio test [18] with the criterion for significance set at $P = 0.05$. Variables that were not statistically significant were dropped by backwards elimination. The final model was obtained by retaining only those factors significantly associated with vertical transmission of HIV-1. Only subjects with all values recorded for all variables were included in the regression analysis (complete subject analysis). No interactions were found. Statistical analyses were performed with the STATA statistical software package (1995 version 4.0; College Station, Texas, USA).

Results

Characteristics of the study population

Four hundred and eighty-five HIV-1-seropositive women delivered 553 live-born children (including 64 siblings and four twin pairs) in the participating obstetric centres during the study period. HIV-1 infection status was established for 434 (78%) children. For 119 children, HIV-1 infection status could not be determined because of the following reasons: 78 (14%) were lost to follow-up (most were younger than 2 months of life when last seen at the medical centres); 29 (5%) died before HIV-1 infection status could be determined (most were younger than 8 months of age and causes of death were not clearly related to HIV-1 infection); and 12 (2%) were refusals (median age, 3 years, and were apparently healthy when contacted by the research team).

Only mother–child pairs for whom the infant's infection status could be determined were included in the analysis. No significant difference was observed between included and excluded mother–child pairs regarding mode of maternal HIV-1 infection, stage of maternal HIV-1 disease, serological evidence of syphilis during pregnancy, mode of delivery, gestational age at birth and breastfeeding. Excluded mothers were significantly more likely to be of mixed race and to have no live-in partner during pregnancy (Table 1). There was no significant difference between included and excluded groups concerning parity, number of lifetime sexual partners, household income, maternal age, alcohol drinking during pregnancy, third trimester amniocentesis or DMR. However, excluded mothers were less educated, had fewer or no antenatal visits, or birthplace outside Sao Paulo State (data not shown).

Sixty-nine of the 434 infants with known infection status were HIV-1-infected (overall vertical transmission risk 16%; 95% CI, 13–20). Nineteen children were defined as infected based on clinical criteria only; all others had serological evidence of HIV-1 infection. Thirty-three (48%) of the 69 HIV-1-infected children had already died from AIDS by the time of data collection, 18 of whom died before 1 year of life. Three out of the four twin pairs were uninfected; in the fourth pair, the first-born child was infected and the second-born was uninfected. The lower and upper estimates of transmission risk, assuming that infants with unknown HIV-1 infection status were either all uninfected or all infected, were 12% (95% CI, 10–16) and 34% (95% CI, 30–38), respectively. Differences in transmission risks across centres and year of birth were not statistically significant.

Table 1. Selected characteristics of the mother–child pairs enrolled in the study, according to whether they were included in the statistical analyses.

Characteristics	No. (%) mother–child pairs		
	Included	Not included	*P*
Racial group			
White	227 (53)	48 (45)	
Mixed race	124 (29)	48 (45)	0.004
Black	79 (18)	11 (10)	
Mode of maternal HIV-1 infection			
Injecting drug use	179 (42)	52 (52)	
Partner of high-risk man	224 (53)	43 (43)	0.19
No identified risk	24 (6)	5 (5)	
Advanced maternal HIV-1 disease at delivery*			
No	372 (86)	103 (88)	
Yes	60 (14)	14 (12)	0.59
Serological evidence of syphilis during pregnancy†			
No	338 (90)	97 (88)	
Yes	38 (10)	13 (12)	0.61
Mother living with partner at time of delivery			
No	64 (15)	55 (47)	
Yes	370 (85)	63 (53)	< 0.001
Mode of delivery			
Caesarean section	150 (35)	41 (34)	
Vaginal	279 (65)	78 (66)	0.92
Gestational age at birth (weeks)			
38–43	182 (68)	81 (69)	
36–37	86 (21)	23 (20)	0.83
28–35	44 (11)	13 (11)	
Breastfeeding			
Never	264 (61)	64 (55)	
Ever‡	168 (39)	52 (45)	0.25

*Advanced maternal HIV-1 disease was defined as the presence of AIDS or HIV-1-related diseases during pregnancy or death from AIDS within 2 years after delivery. †Serological syphilis was defined as a quantitative Venereal Disease Research Laboratory test ≥ 8 during pregnancy or at delivery. ‡Mean duration, 98 days; median, 30 days; range, 1 day to 2 years. Percentages may not total 100% because of rounding.

Table 2. Mother-to-child transmission of HIV-1 according to maternal factors.

	Univariate analysis		Multivariate analysis		
Maternal factors	No. (%) infected	95% CI	Adjusted OR*	95% CI	*P*
Maternal age at delivery (years)					
14–19	10 (13)	6–22	1.0		
20–25	28 (15)	10–21	1.2	0.5–3.2	
26–30	21 (20)	13–28	2.1	0.8–5.5	
31–39	10 (16)	8–27	1.1	0.4–3.6	0.46†
Racial group					
White	41 (18)	13–24	1.0		
Mixed race	21 (17)	11–25	0.9	0.4–1.7	
Black	5 (6)	2–14	0.3	0.1–0.9	0.04
Advanced maternal HIV-1 disease					
No	48 (13)	10–17	1.0		
Yes	21 (35)	22–43	4.5	2.1–9.5	< 0.001
Serological evidence of syphilis					
No	57 (17)	13–21	1.0		
Yes	3 (8)	2–21	0.4	0.1–1.5	0.15
Number of household goods					
0–3	39 (16)	11–21	1.0		
≥ 4	15 (11)	7–18	0.6	0.3–1.3	0.2
Alcohol drinking during pregnancy					
No	38 (13)	9–17	1.0		
Yes	19 (21)	13–31	1.6	0.8–3.3	0.22
Number of lifetime sexual partners					
1–3	9 (6)	3–11	1.0		
4–10	16 (16)	10–25	1.8	0.7–4.7	
> 10	11 (15)	8–25	2.7	0.9–7.4	0.06†

*Odds ratio (OR) adjusted for stage of maternal HIV-1 disease, breastfeeding, racial group, child's Rhesus blood type and third trimester amniocentesis. †Test for trend. CI, Confidence interval.

Maternal factors and HIV-1 transmission

Maternal age at delivery, serological evidence of syphilis during pregnancy, number of household goods and alcohol drinking during pregnancy were not significantly associated with HIV-1 infection status in infants. A trend towards higher transmission risk associated with increasing number of lifetime sexual partners was observed but did not reach statistical significance (*P* test for trend = 0.06). Advanced maternal HIV-1 disease (defined by presence of AIDS or HIV-related clinical manifestations during pregnancy or death from AIDS within 2 years of delivery) was strongly and independently associated with increased risk of vertical transmission of HIV-1 (OR, 4.5; 95% CI, 2.1–9.5). Black mothers were significantly less likely to transmit HIV-1 than white mothers (OR, 0.3; 95% CI, 0.1–0.9; Table 2).

Transmission was not associated with other maternal factors, including parity, mode of acquisition of HIV-1, history of use of injecting or non-injecting drugs, cigarette smoking during pregnancy, having a previous child with AIDS, unprotected sex with an HIV-positive partner during pregnancy, presence of genital warts or detection of hepatitis B surface antigen during pregnancy, and maternal ABO or Rhesus blood types. In addition, none of the socioeconomic covariates measured (number of visits to an antenatal care unit, number of years of schooling, household income, availability of piped water and sewage) were significantly associated with HIV-1 infection in children.

Obstetric factors

The risk of vertical transmission was similar for vaginally and operatively (by caesarean section) delivered infants. In the multivariate analysis, prolonged DMR (> 25 h) was associated with a substantial increase (OR, 3.9; 95% CI, 1.0–14.7) in the risk of infection in the infant, although the trend between DMR and HIV-1 transmission did not reach statistical significance (*P* test for trend = 0.06). Third trimester amniocentesis was strongly associated (OR, 4.1; 95% CI, 1.2–13.5) with increased risk of vertical transmission of HIV-1 after adjustment was made for stage of maternal HIV-1 disease, racial group, breastfeeding and child's Rhesus blood type (Table 3).

Vertical transmission risk was not associated with duration of labour, indications for caesarean section or with whether caesarean section was performed electively (prior to labour regardless of DMR). Among women who gave birth vaginally, there was no significant difference in risk according to performance of episiotomy or use of forceps.

Neonatal and post-natal factors

There were no significant differences in risk of transmission by the child's gender, ABO blood type, gross placental abnormalities or mother–child Rhesus or ABO incompatibility. In the multivariate logistic regression analysis, there was a 2.5-fold increase in risk of transmission for children who were Rhesus-negative (*P* = 0.03). There was a non-significant trend towards

Table 3. Mother-to-child transmission of HIV-1 according to obstetric factors.

	Univariate analysis		Multivariate analysis		
Obstetric factors	No. (%) infected	95% CI	Adjusted OR*	95% CI	*P*
Mode of delivery					
Caesarean section	28 (19)	13–26	1.0		
Vaginal	41 (15)	11–19	0.9	0.4–1.7	0.67
Duration of membrane rupture (h)					
< 1	22 (15)	9–21	1.0		
1–6	26 (17)	11–24	1.3	0.6–3.0	
7–24	9 (16)	8–29	1.7	0.6–4.7	
≥ 25	6 (33)	13–59	3.9	1.0–14.7	0.06†
Third trimester amniocentesis					
No	63 (15)	12–19	1.0		
Yes	6 (40)	16–68	4.1	1.2–13.5	0.03

*Odds ratio (OR) adjusted for stage of maternal HIV-1 disease, breastfeeding, racial group, child's Rhesus blood type and third trimester amniocentesis. †Test for trend. CI, Confidence interval.

Table 4. Mother-to-child transmission of HIV-1 according to neonatal and post-natal factors.

	Univariate analysis		Multivariate analysis		
Neonatal/post-natal factors	No. (%) infected	95% CI	Adjusted OR*	95% CI	*P*
Gestational age at birth (weeks)					
38–43	39 (14)	10–18	1.0		
36–37	18 (21)	13–31	1.4	0.7–3.0	0.06†
28–35	11 (25)	13–40	2.3	0.9–5.6	
Child's Rhesus blood type					
Positive	43 (14)	13–26	1.0		
Negative	13 (31)	11–19	2.5	1.2–5.5	0.03
Breastfeeding					
Never	33 (13)	9–17	1.0		
Ever	36 (21)	15–28	2.2	1.2–4.2	0.01

*Odds ratio (OR) adjusted for stage of maternal HIV-1 disease, breastfeeding, racial group, child's Rhesus blood type and third trimester amniocentesis. †Test for trend. CI, Confidence interval.

increased risk with prematurity (*P* test for trend = 0.06). Children who were ever breastfed had a 2.2-fold increased risk of being infected (95% CI, 1.2–4.2; Table 4).

Discussion

The overall risk of vertical transmission of HIV-1 in this study population of Sao Paulo State was 16% (95% CI, 13–20) and is likely to reflect the distribution of risk factors in this population, and in particular the relatively low proportion of mothers with advanced HIV-1 disease (12%) and breastfeeding (39%). Potential for bias included loss to follow-up and the death of children before determination of their HIV-1 infection status. Most children born to women who were lost to follow-up or who refused to participate in the study were probably uninfected, otherwise they would have eventually been in contact with the health services for follow-up of HIV-1-related diseases [19]. On the other hand, those children who died before their HIV-1 infection status could be ascertained were more likely to be infected. Therefore, overall it appears unlikely that loss to follow-up and death of children with unknown HIV-1 infection status would have substantially affected the precision of our estimates.

Recent studies that analysed immunological and virological markers confirmed that progression of maternal disease is associated with high viral load and immunosuppression leading to increased vertical transmission risk [3–6,10,20]. In our study, because CD4 lymphocyte counts and viral load were unavailable, clinical markers of disease progression were used to classify disease stage. Advanced maternal HIV-1 disease was strongly associated with a high risk of vertical transmission, in accordance with several other reports that used similar parameters [4,5,7]. Syphilis has been identified as a cofactor in female-to-male sexual transmission of HIV-1 [21,22] and it has been postulated that syphilis coinfection could also influence vertical transmission. However, as noted by us and in several other studies [3,8,23,24], positive syphilis serology during pregnancy was not related to transmission of HIV-1 from mother to child. Bearing in mind that antibody detection is an unsatisfactory marker for active syphilis, our finding and that of others suggests either that syphilis does not affect vertical HIV-1 transmission, or that the studies were insufficient to detect a small effect.

An association between maternal sexual behaviour during pregnancy and transmission of HIV-1 to the infant has been reported in several studies [20,24–27], but the reasons why this would occur remain unclear and

residual confounding effect of maternal and obstetric factors cannot be fully ruled out [28]. Our data refer to the number of lifetime sexual partners rather than sexual behaviour during pregnancy, and increasing number of partners was weakly associated with a higher risk of vertical transmission.

By avoiding contact between the child and birth canal, caesarean section could potentially reduce the risk of transmission as noted in some studies [5,7,29], but not in the current study nor in other cohorts [3,4,6,30]. In addition, no association between intrapartum events such as forceps and episiotomy and an increased risk of infant's infection was observed in this or other studies [3,5,6]. However, our observation of a fourfold increase in transmission with third trimester amniocentesis (which was performed to assess maturity of fetal lungs) is consistent with a recent report by Mandelbrot *et al.* [3] in which a twofold increase in transmission risk was associated with needling procedures or amnioscopy during pregnancy. Findings regarding the effect of DMR on transmission have been conflicting, with some studies showing an association [3,20,30–32] whereas others have not [5,8,25,33]. Comparisons between studies are difficult because different strata have been used to categorize DMR, and the confounding effect of other parameters such as duration of labour, types of caesarean section (elective and emergency), length of gestation and stage of maternal HIV-1 disease has not always been taken into account. By the same token, prematurity has not been consistently associated with higher transmission risk [7,10,23,25,34]. However, it is generally accepted that shorter gestation and DMR are associated with increased risk of vertical transmission of HIV-1.

Compatibility between maternal and fetal Rhesus blood type did not affect the risk of vertical transmission, but children who were Rhesus-negative were at higher risk of HIV-1 infection than children who were Rhesus-positive. Some studies have not found an association between race and risk of vertical transmission of HIV-1 [5,10,35], although in our study black infants were at lower risk of infection than white infants. These findings may have occurred due to chance since multiple comparisons were performed. Alternatively, Rhesus blood type and race could be markers for genetic differences. Although the association between genetic determinants and increased susceptibility to vertical transmission is plausible, it has not been confirmed because of the small numbers in the few published studies [36,37]. A study from Scotland found that certain human leukocyte antigen haplotypes were more frequent in HIV-1-infected than in HIV-uninfected infants [36]. An American study reported that a high-risk allele in the infant was associated with increased vertical transmission of HIV-1 [37]. The mechanisms for an increased risk of transmission associated with Rhesus-negative blood type and race are speculative and these findings need to be confirmed.

It is now well established that transmission of HIV-1 through breastfeeding does occur independently of stage of maternal HIV-1 disease and our findings are consistent with other studies [4,38,39]. Risk of transmission may depend upon other factors related to breastfeeding such as its duration, other feeding practices (mixing of formula or cereals), presence of cracked nipples, child's oral pathology and teething. The understanding of these factors is critical for the establishment of recommendations for HIV-1-infected women in settings where the protective effects of breastmilk on infant survival and nutritional status outweigh the potential for HIV-1 transmission through breastfeeding.

In conclusion, the early identification of HIV-1 infection in pregnant women is crucial for the implementation of strategies to prevent transmission of HIV-1 from mother to child. Recommendations to reduce transmission of HIV-1 to infants should include avoidance of amniocentesis and invasive antenatal procedures that can potentially cause iatrogenic infection of the fetus. Health-care providers should help HIV-1-infected mothers to understand and to balance the potential for transmission of HIV-1 through breastmilk with the known benefits of breastfeeding. Ultimately, mothers can make an informed decision not only about antiretroviral therapy, but also about whether to initiate breastfeeding. Support programmes for alternatives to breastmilk should be provided for women who opt not to breastfeed and who cannot afford artificial feeding.

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Appendix

Sao Paulo Collaborative Study for Vertical Transmission of HIV-1

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