HIV-1 Viremia During the First 28 Weeks of Pregnancy is Not Associated With Mother-to-Child Transmission

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It is currently recommended that antiretroviral prophylaxis to prevent mother-to-child transmission (MTCT) of HIV be initiated at 14 weeks of gestation. However, the relevance of early-gestation HIV viral load level for intrauterine MTCT is unknown. The objective of this study was to determine the relationship between prenatal maternal viral load and intrauterine MTCT. Records of HIV-infected pregnant women in two centers in Brazil, from 1999 to 2004 were analyzed. Three pregnancy periods were considered: earlier than 14 weeks, 14 to 27 $^6/_7$ weeks, and 28 weeks of gestation or more. Peripartum HIV exposure was also computed. Maximum viral load in each period was the measure of HIV exposure. Four hundred fifty-seven HIV-infected pregnant women were evaluated, but 53 were excluded. The MTCT rate was 0.49% (2/404–95% confidence interval (CI_{95}) = 0.14–1.79%). Newborns were not breast-fed. Median viral load for the earlier-than-14-week period was 9,900 copies/mL (P_{25-75} 1,000–50,775 copies/mL), 8,350 copies/mL (P_{25-75} 707–42,000 copies/mL) for the 14 to 27 $^6/_7$-week period, and 435 copies/mL (P_{25-75} 90–7,775 copies/mL) after the 28-week period. The peripartum median viral load was 400 copies/mL (P_{25-75} 80–500 copies/mL). MTCT in mothers with VL > 1,000 copies/mL during the first 14 weeks (0.67%, 2/298) was not different from those with VL =1,000 copies/mL (0.0%, 0/96, P=1). Analogously, in the 14 to 27 $^6/_7$-week period, MTCT was similar in groups with VL higher (0.68%, 2/292) or lower (0%, 0/106) than 1,000 copies/mL (P=1). Regarding VL >1,000 copies/mL at 28-weeks-or-later and at peripartum periods, MTCT rates were 1.15% (2/173, P = 0.18) and 2.8% (2/71, P = 0.03), respectively. Intrauterine transmission does not seem to be influenced by HIV viremia during the first 28 weeks of pregnancy.
Key Words: HIV-1, disease transmission, vertical transmission, antiretroviral therapy, highly active, Brazil, viral load.

According to the United Nations Program on HIV-AIDS/World Health Organization, approximately five million new HIV infections occurred worldwide in 2004. Among those infected, 40% were women of childbearing age, resulting in approximately 700,000 children being infected [1]. As of June 2004, 310,245 AIDS cases had been reported among Brazilians over the age of 13. Women accounted for 89,302 cases, 87.6% of which were sexually transmitted [2]. According to the Brazilian Ministry of Health, the estimated prevalence of HIV infection among pregnant women was 0.47% in 2000 [3].

In the absence of effective prophylactic intervention, approximately 25% of mothers transmitting HIV infection to their child will do so during pregnancy, and 75% will transmit the virus during the perinatal period [4]. In the case of breastfeeding, the risk of transmission can increase 14% to 29% [5,6]. The impact of known risk factors for HIV transmission during the perinatal period (protracted labor, membrane rupture for more than four hours and especially perinatal HIV viral load) is clearly reduced by antiretroviral therapy during pregnancy and by elective cesarean section (C-section) [7-11]. As a result of these interventions, perinatal mother-to-child transmission (MTCT) of HIV has been significantly reduced. Consequently, intrauterine transmission has become the predominant mode of MTCT, accounting for up to 80% of all cases [12]. Initiating antiretroviral therapy at week 28 of gestation [13] can reduce intrauterine MTCT, which has been correlated with higher prenatal maternal viral load, suboptimal antiretroviral therapy, and low birth weight [12]; although data regarding these and other relevant risk factors for intrauterine MTCT are scarce. Therefore, effective interventions cannot be devised until the pathogenic mechanisms of intrauterine transmission are better understood.

Despite the fact that most guidelines for antiretroviral therapy in pregnancy recommend it [14,15], the available evidence does not indicate that prophylactic antiretroviral therapy initiated by week 14 of gestation affords more protection than that initiated at week 28 [13,16]. On the other hand, it has been demonstrated that zidovudine monotherapy started after 34 weeks of gestation has no impact on intrauterine MTCT [13,17]. In view of these facts, the objective

of our study was to analyze the relationship between prenatal exposure to HIV (particularly during the first 28 weeks of gestation) and MTCT of HIV.

Material and Methods

All HIV-positive pregnant women receiving prenatal care between January 1999 and June 2004 at two referral centers, the Universidade Federal de São Paulo (UNIFESP, Federal University of Sao Paulo) Hospital São Paulo and the Hospital Ipiranga, both located in Sao Paulo city, Brazil, were included in this study. Clinical and laboratory records were reviewed retrospectively. Medical care, laboratory tests, and drugs are provided without charge at both centers. Lactation was routinely inhibited and breastfeeding discouraged. During the first 72 hours after delivery, and 30 days thereafter, the HIV viral load of the child was routinely assessed. At one year of age or more, infants were submitted to HIV ELISA testing. Non-MTCT was defined as an undetectable HIV viral load in at least two tests, more than 30 days apart [18].

To assess the influence that HIV exposure during pregnancy has on MTCT, gestation was divided into three periods: = 14 weeks; 14-27 weeks; and ≥ 28 weeks. In addition, the perinatal period was defined as the last four weeks preceding delivery. The highest recorded viral load in each period was considered the level of HIV exposure during that period. In antiretroviral-naïve patients, viral load assessed before the initiation of treatment was computed in the analysis as HIV exposure up to that moment. Similarly, for pregnant women on antiretroviral therapy before conception, HIV exposure was computed as the viral load assessed at the time of the first prenatal care appointment. It was assumed that the observed viral load had been constant during previous periods. For those women who began to receive antiretroviral therapy during pregnancy, presenting an undetectable viral load at the first prenatal visit and lacking a baseline viral load determination, HIV exposure for previous periods was listed as missing data. However, whenever HIV viral load was > 400 copies/mL at the first prenatal visit, despite the use of antiretroviral agents, that value was used to compute HIV exposure for the previous periods. For pregnant women for whom the antiretroviral regimen was altered during more than one week after the last perinatal viral load assessment, HIV exposure for that period was listed as missing data.

For each gestation period, HIV exposure was categorized as < 1,000 copies/mL or ≥ 1,000 copies/mL. The viral loads of the mothers who transmitted HIV were analyzed in comparison to the viral loads of non-transmitters in each of the above-mentioned gestation periods in order to assess the potential relevance of viremia for transmission in relation to the gestation period. This study was approved by the UNIFESP Ethics Committee and was in compliance with applicable Brazilian and international ethical standards.

Descriptive analyses were used to characterize the population. Correlations between HIV viral loads and MTCT were determined using Fisher's exact test. All analyses were performed with SPSS for Windows, version 10.0 (SPSS, Inc., Chicago, IL, USA). Values of $P < 0.05$ were considered statistically significant.

Results

From January 1999 to June 2004, 457 HIV-positive pregnant women were treated. Of those 457, 53 were excluded from the final analyses: 31 because they dropped out of prenatal care; seven due to abortion/miscarriage; eight because of fetal death; and seven because their patient records lacked data regarding HIV viral load or antiretroviral therapy, although no HIV transmission occurred in these latter seven cases. Therefore, the final sample consisted of 404 women, 141 (34.9%) of whom became aware of their HIV status during the current pregnancy, and 263 (65.1%) of whom were not on antiretroviral therapy when they conceived. The mean age was 28 years (range, 14-46 years), and median gestational age at the first prenatal care visit was 20 weeks (range, 6.5-38 weeks). Prenatal care began at 28 weeks of gestation or later in 122 (30.2%) of the women, and after 35 weeks in 11 (2%).

Initial median CD4+ T-cell counts and HIV viral load in pregnancy were 372 cells/mm^3 (range, 2–1,423 cells/mm^3) and 8,400 copies/mL (range, < 80–1,900,000 copies/mL), respectively. Highly active antiretroviral therapy (HAART) during pregnancy was used by 378 (93.6%) of the women: nevirapine by 191 (47.4%), and protease inhibitor by 187 (46.2%). Exclusive zidovudine monotherapy and double-nucleoside analog therapy were prescribed to, respectively, seven (1.7%) and 16 (4%) of the women. The remaining three pregnant women (0.7%) received zidovudine monotherapy at delivery. Of the 391 cases for which perinatal laboratory test results were available, HIV viral load was below the level of detection in 282 (72.1%).

Elective C-section was performed in 277 (68.5%) of the 404 cases investigated, urgent C-section in 73 (18.1%), and vaginal delivery in 54 (13.4%). Premature delivery occurred in 58 (14.4%) of the cases.

Among the 404 women evaluated, the rate of MTCT was only 0.49% (2/404; 95% confidence interval (CI): 0.14–1.79%). The first case of transmission occurred in 1999 in a woman who had started antiretroviral therapy long before becoming pregnant. At the time of conception, the woman was on zidovudine and lamivudine. At the first prenatal visit (at week 22 of gestation), her HIV viral load was determined to be 25,000 copies/mL, and a CD4+ count of 120 cells/mm^3 was recorded. Consequently, HAART (nelfinavir, stavudine and didanosine) was initiated. Her HIV viral load was maintained at approximately 25,000 copies/mL throughout gestation and the elective C-section was done at 38 weeks. No data regarding genotyping or adherence were available. Since the offspring

viral load was only measured one month after birth, it was not possible to determine whether transmission was intrauterine or not. The second case occurred in 2002 in a woman who was on zidovudine, lamivudine and indinavir before conception. She began prenatal care at 23 weeks of gestation, at which time her HIV viral load was 3,400 copies/mL, and her CD4+ count was 376 cells/mm^3. Despite several attempts to determine the genotype, amplification was unsuccessful. At 31 weeks of gestation, the patient was put on lopinavir/r, didanosine and stavudine. Her HIV viral load remained > 1,000 copies/mL throughout gestation and was 1,510 copies/mL at the time of C-section (at 38 weeks). In this case, HIV intrauterine transmission was confirmed. The relevance of HIV viral load to HIV transmission for the two infected offspring was analyzed for each of the pregnancy periods: < 14 weeks, from 14-28 weeks, and > 28 weeks.

Median HIV viral load during the 0 to 14 weeks of gestation period was 9,900 copies/mL (interquartile range, 1000–50,775 copies/mL). The MTCT rate among women with HIV viral loads ≥ 1,000 copies/mL during this period was 0.67% (2/298; 95% CI: 0.18–2.41), compared with 0% among those with HIV viral loads < 1,000 copies/mL (P = 1). In the 14 to 28 week period, the median HIV viral load to which fetuses were exposed was 8,350 copies/mL (interquartile range, 707–42,000 copies/mL). For this period, there was also no significant difference in MTCT rates between women with HIV viral loads ≥ 1,000 copies/mL (0.68%; 2/292; 95% CI: 0.19–2.45%) and the remaining women (0%; P = 1). At 28 weeks or more of gestation, the median HIV viral load to which fetuses were exposed was 435 copies/mL (interquartile range, 90–7775 copies/mL), During this period, the MTCT rate among those with HIV viral loads = 1,000 copies/mL was 1.15% (2/173; 95% CI: 0.32–4.12) and 0% among those with < 1,000 copies/mL (P = 0.18). The median HIV viral load to which fetuses were exposed in the perinatal period was below the lower limit of detection of 400 copies/mL (interquartile range, 80–500 copies/mL). The perinatal MTCT rate was 2.81% (2/71; 95% CI: 0.34–9.8) among women with ≥ 1,000 copies/mL and 0% among those with < 1000 copies/mL (P = 0.032).

For the 0 to 14 weeks of gestation period, HIV viral load data were missing for 10 women, who were therefore unaccounted for in the analysis of this period. There was a similar lack of data for six women in relation to the 14 to 28 week period. For the perinatal period, HIV viral load data were not collected for 13 patients.

Discussion

We found that exposure to viral loads > 1,000 copies/mL in the first 28 weeks of gestation is not associated with increased risk of MTCT of HIV. Neither the probability of achieving a perinatal HIV viral load < 1,000 copies/mL nor the risk of MTCT were affected by postponing HAART initiation to after 28 weeks of gestation.

The rate of MTCT in our study was 0.49% (2/404), whereas the rate in Brazil as a whole, during 2000 to 2002, was 6.8%, ranging from 12.3% in the northeastern region to 5.5% in the south [19]. Such a striking difference could be attributed to the multidisciplinary approach of the facilities participating in our study, as well as to the use of HAART, as opposed to zidovudine monotherapy, the latter still being recommended in the Brazilian guidelines for antiretroviral therapy administered to pregnant women [14]. In the Netherlands, exclusive use of HAART after 21 weeks of gestation resulted in a similar MTCT rate (0.7%; 2/267) [20].

The relevance of mean prenatal viral load for MTCT was stressed by Magder et al. [12] in the Women and Infants Transmission Study cohort. However, averaging viral loads determined throughout pregnancy precludes any conclusion about the contribution of viral load exposure in each gestation period, i.e. first *versus* third trimester. In our study, HIV viral loads during the first 14 weeks of gestation were < 1,000 copies/mL in only 24.4% (96/394) of the women evaluated, > 100,000 copies/mL in 14% (55/394) and above the upper detection limit of 750,000 copies/mL in 1% (4/394). For the same period, the MTCT rate among women with viral loads > 1,000 copies/mL was not significantly higher than that seen among women with viral loads = 1,000 copies/mL. During this period, there were six miscarriages among women; they had a median viral load of 5,350 copies/mL, ranging from undetectable to 390,000 copies/mL. Even assuming that all miscarriages were due to HIV infection, this could not be explained on the grounds of viral load alone.

In the 14 to 28 week period, there was also no significant difference between the rate of MTCT among women presenting HIV viral loads ≥ 1,000 copies/mL and that among those with viral loads < 1,000 copies/mL. During this period, viral loads were > 1,000 copies/mL in 294 women (73.9%) and > 100,000 copies/mL in 52 (13%).

The two women who transmitted HIV to their offspring had viral loads in the first 28 weeks of only 3,500 and 25,000 copies/mL, respectively, and both had peripartum viral loads of 1,000 to 24,000 copies/mL. These women were already on HAART at the time of conception and HAART was continued throughout pregnancy.

These data support the concept that the introduction of antiretroviral prophylaxis during the first 28 weeks of gestation does not have an impact on MTCT. Nevertheless, guidelines for the prevention of MTCT recommend that antiretroviral therapy be initiated after 14 weeks of gestation [14,15]. The timing of antiretroviral therapy initiation was established in 1994, on the basis of the Pediatric AIDS Clinical Trial Group (PACTG) 076 study [21]. However, closer examination of the PACTG 076 data reveals no evidence to support the conclusion that antiretroviral therapy initiated at 14 weeks of gestation is more effective than that initiated at 26 weeks. In the PACTG 076 protocol, prior to randomization, pregnant women were stratified according to gestational age at study

entry (14 to 26 weeks or 26 to 34 weeks). One conclusion of the PACTG 076 study was that MTCT rates did not differ between women in whom zidovudine had been started before week 26 of gestation and those in whom it had been started later. In the multivariate analysis, including timing of antiretroviral therapy initiation, the only variable that correlated significantly with MTCT was the use of zidovudine. In fact, intrauterine transmission in the PACTG 076 study was 2% [16], similar to the 1.6% found by Lallement et al. [13], who used zidovudine alone starting at 28 weeks of gestation. Therefore, intrauterine transmission does not seem to be reduced by initiating antiretroviral therapy early in pregnancy. It is notable that use of antiretroviral therapy as prophylaxis against MTCT for longer periods can increase both non-adherence to the regimen and health care system expenditures. In addition, the extent of genotoxicity, as a result of long-term fetal exposure to antiretroviral therapy and the consequent incorporation of nucleoside analogs into the offspring DNA [22], is still unknown.

In contrast, perinatal viral load > 1,000 copies/mL was found to be a clear risk factor for MTCT (P = 0.03). Viral load at delivery has also been shown to be highly predictive of MTCT in a variety of settings [11,23,24], Shapiro et al. [25] demonstrated that HAART significantly reduced MTCT among pregnant women; the percentage women with viral loads < 1,000 copies/mL was significantly lower among those on HAART than among those on zidovudine monotherapy.

In conclusion, we suggest that intrauterine transmission is not influenced by HIV viremia during the first 28 weeks of gestation. We can therefore argue that the current recommendation to initiate antiretroviral prophylaxis at 14 weeks of gestation may indeed be doing fetuses more harm than good.

Acknowledgments

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