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Risk of congenital cytomegalovirus infection among HIV-exposed uninfected infants is not decreased by maternal nelfinavir use during pregnancy

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Abstract

Background—Congenital cytomegalovirus (cCMV) infection is common among infants born to HIV-infected women. Nelfinavir (NFV), an antiretroviral drug that is safe during pregnancy, inhibits CMV replication *in vitro* at concentrations that standard doses achieve in plasma. We hypothesized that infants born to women receiving NFV for prevention of mother-to-child transmission of HIV (PMTCT) would have a reduced prevalence of cCMV infection.

Methods—The prevalence of cCMV infection was compared among HIV-uninfected infants whose HIV-infected mothers either received NFV for ≥4 weeks during pregnancy (NFV-exposed) or did not receive any NFV in pregnancy (NFV-unexposed). CMV PCR was performed on infant blood samples collected at <3 weeks from birth.

Results—Of the 1,255 women included, 314 received NFV for ≥4 weeks during pregnancy and 941 did not receive any NFV during pregnancy. The overall prevalence of cCMV infection in the

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Conflicts of interest

No authors declare a conflict of interest.

infants was 2.2%, which did not differ by maternal NFV use. Maternal CD4 T cell counts were inversely correlated with risk of cCMV infection, independent of the time NFV was initiated during gestation. Infants with cCMV infection were born 0.7 weeks earlier (p=0.010) and weighed 170 grams less (p=0.009) than uninfected infants.

Conclusion—Among HIV-exposed uninfected infants, cCMV infection was associated with adverse perinatal outcomes. NFV use in pregnancy was not associated with protection against cCMV. Safe and effective strategies to prevent cCMV infection are needed.

Keywords

congenital cytomegalovirus infection; nelfinavir; HIV-exposed uninfected infants

Introduction

Congenital cytomegalovirus (cCMV) infection occurs in 0.2 – 2% of all live births in the United States, and is a major cause of deafness and intellectual disability.(1) Studies have reported high rates of cCMV infection among infants born to HIV-infected women,(2–8) which is likely due in part to the impaired control of CMV replication that results from HIV-related immune deficiencies.(4) Maternal antiretroviral use during pregnancy is associated with protection against cCMV infection; however, even with combination antiretroviral regimens, studies suggest that the risk of cCMV is relatively higher compared to rates for HIV-uninfected women.(5, 7) Given the prevalence and morbidity of cCMV, effective preventive strategies would be of substantial clinical benefit to infants' health.

The use of antiviral drugs to prevent cCMV infection is limited by the toxicity of available agents. Ganciclovir and its pro-drug valganciclovir are currently the only oral medications approved for treatment of CMV infection, and have carcinogenic, teratogenic, and gonadotoxic effects that preclude their routine use in pregnancy.(9) Non-randomized studies of intravenous immunoglobulin given to pregnant women with primary CMV infection initially showed protective effects, but these results were not confirmed by a randomized controlled trial.(10) The HIV protease inhibitor nelfinavir (NFV) has been shown *in vitro* to inhibit replication of CMV and other herpesviruses at concentrations achieved clinically.(11) This anti-herpesvirus activity was unique to NFV among the broad panel of antiretrovirals tested. The *in vitro* inhibitory concentrations of NFV against CMV were found to be in the low micromolar range (IC_{50}=4.4 *uM*), and comparable to ganciclovir against susceptible CMV isolates.(12) The anti-CMV activity of NFV raises the possibility that antiretroviral regimens that contain NFV may reduce cCMV infection. NFV was deemed particularly attractive as a potential preventive agent for cCMV infection given that it is already approved for the treatment of HIV infection and it has been used extensively for the prevention of perinatal HIV transmission.(13) NFV has an excellent safety profile, and is relatively inexpensive. Therefore, we tested the hypothesis that NFV can reduce the risk of *in utero* CMV infection.

Methods

Cohort

Data and specimens were obtained from 2 prospective cohorts studied by the Pediatric AIDS Clinical Trials Group (PACTG) and International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network; PACTG 316(14) and P1025 studies.(15) PACTG 316 was a multicenter, randomized, double-blind trial of 2-doses of intrapartum/newborn nevirapine compared with placebo to reduce perinatal transmission of HIV from women on standard ARV therapy between 1997 and 2000. Of the 1,270 women-infant pairs recruited in the US, Europe, Brazil and the Bahamas between 1997 and 2000,(14) the current study included only the 661 women-infant pairs from the US. Another 594 women-infant pairs were drawn from the P1025 study, a US-based observational study designed to assess the use and safety of antiretroviral drugs and other interventions for HIV-infected pregnant women and their infants. In both studies NFV was prescribed as part of clinical care, and not part of a randomized regimen. All procedures followed were in accordance with the ethical standards of the responsible human subjects protection committees and with the Helsinki Declaration of 1975, as revised in 2000.

Inclusion criteria for the current study included all women-infant pairs with a cryopreserved newborn blood specimen (plasma or peripheral blood mononuclear cells (PBMC)) collected at <3 weeks from birth and a history of maternal ARV use. Infants whose mothers received NFV for ≥4 weeks during pregnancy were in the NFV-exposed group and those whose mother did not receive NFV during pregnancy were in the unexposed group. HIV-infected infants were excluded due to the potential interaction with risk of CMV infection.(3)

Demographic and treatment data were analyzed: maternal data included age, race and ethnicity, stage of HIV disease,(16) antiretroviral use, gestational week at initiation of NFV and other antiretrovirals, earliest and latest maternal CD4 T cell counts and HIV plasma RNA loads in pregnancy, and mode of delivery. Infant data included sex, gestational age, weight at delivery, and infant HIV infection status. For both PACTG 316 and P1025, infant gestational age was estimated at the baseline pregnancy visit, and modified based on ultrasound data and physical exam at delivery.

CMV PCR testing

cCMV infection was determined by detection of CMV DNA in newborn blood specimen using real-time PCR at the University of Washington Virology Laboratory.(17) DNA was extracted from 0.4 mL of plasma or $\geq 10^6$ PBMC. Detection of ≥50 CMV genome copies per mL of plasma or ≥5 CMV genome copies per reaction for PBMC was considered positive. All runs of the assay included positive and negative controls, and each reaction was spiked with an internal control to detect inhibition of PCR. Study personnel were blinded to the NFV exposure group assignment until all testing was completed.

Statistical analyses

The proportion of infants with cCMV infection in the NFV-exposed and the NFV-unexposed groups was compared using Fisher’s exact test. Among infants with cCMV infection, we

compared CMV plasma viral load by NFV exposure using the Wilcoxon test. Other predictors of cCMV infection were also explored using Fisher's exact test and Wilcoxon tests as appropriate. Multivariable logistic regression models were constructed to explore the independent associations of selected covariates with cCMV infection. Due to the low number of events, we used exact methods to estimate adjusted odds ratios (aOR) and 95% confidence intervals (CI).

Results

Characteristics of study subjects

A total of 314 infant subjects from the PACTG 316 and P1025 studies were identified who had a specimen available for CMV testing and whose mothers received NFV for at least 4 weeks during pregnancy (Table 1). Among them, 69 (22%) initiated NFV prior to pregnancy, 36 (11%) during the first trimester, 163 (52%) during the second trimester and 46 (15%) during the third trimester. 85% continued NFV through the time of delivery. There were 941 NFV-unexposed infants whose mothers by design did not receive any NFV. Women not exposed to NFV were more likely to have been diagnosed with HIV prior to the pregnancy (p=0.018), initiated antiretrovirals earlier (p=0.007), and have higher CD4 T cell counts during pregnancy (p=0.043) and at delivery (p=0.006), compared to women who received NFV. NFV-exposed and unexposed women did not differ in HIV viral load during pregnancy or at delivery, gestational age at delivery, delivery mode or their infant's birth weight.

Prevalence of cCMV infection by NFV exposure

A total of 27 infants (2.2%) had cCMV infection (Table 1). The proportion of infants with cCMV infection did not differ significantly between the NFV-exposed and unexposed groups (8/314 (2.5%) vs. 19/941 (2.0%); p=0.653). CMV was detected in plasma specimens significantly more often than in PBMC (22/764 (2.9%) vs. 5/491 (1.0%); p=0.028). The proportion of infant PBMC samples tested for CMV did not differ significantly between NFV exposure groups (exposed, 117/314 (37%) vs. unexposed, 374/941 (40%); p=0.435). Of those with CMV detected in plasma (N=22), there was no significant difference in the median number of genome copies/mL between NFV exposure groups (Figure 1; PBMC were excluded given the different units and small number of PBMC specimens in which CMV was detected).

Clinical characteristics by cCMV infection status

Maternal and infant characteristics by cCMV infection status are shown in Table 2. In the univariable analysis, mothers whose first CD4 T cell counts in pregnancy was <200 cells/mm^3 were more likely to have infants with cCMV infection than mothers with higher CD4 T cell counts. Mothers of infants with cCMV infection initiated antiretroviral therapy significantly later during gestation compared to mothers of CMV-uninfected infants (p=0.037). However, significant differences were not observed in the time that maternal NFV was initiated or duration of fetal exposure to NFV by cCMV infection status. Infants with cCMV infection had a lower median birth weight (2.87 vs. 3.04 kg; p=0.009) and were born a median gestation that was 5 days shorter compared to uninfected infants (p=0.010). When adjusted for first CD4 count <200 cells/μL in pregnancy, the odds of preterm birth

was not higher in those with cCMV infection compared to those without cCMV infection (aOR 2.32, 95% CI 0.81, 5.81; p=0.116). In the univariable analysis, NFV exposure was not associated with cCMV infection by (OR 1.27; 95% CI 0.48, 3.07; p=0.710; Table 3). Furthermore, there was no association between NFV exposure and cCMV infection in two multivariable models that adjusted for timing of antiretroviral initiation plus either specimen type or timing of antiretroviral initiation (Table 3).

Discussion

In this observational cohort study, maternal NFV during gestation was not associated with a reduction in the probability of cCMV infection, nor with the quantity of CMV detected in plasma of infected infants. While NFV inhibits CMV replication in vitro,(11) the lack of a detectable protective effect of NFV may have been due to low plasma concentrations of NFV during the pregnancy(18) or insufficient transport of NFV across the placenta.(19) We hypothesized that NFV might suppress maternal CMV viremia and viral replication in the placental cytotrophoblasts, thereby reducing the likelihood of virus crossing the placenta to the fetus. However, it is possible that transplacental passage of the antiviral is required for effective antiviral prophylaxis against cCMV infection. NFV-resistant CMV is also possible, though there may be a high barrier to resistance as NFV appears to inhibit herpesvirus replication by targeting the host cell rather than the virus.(20, 21)

Given that NFV use was not randomly assigned to women in this study, the highly variable timing in initiation of NFV and duration of NFV exposure may have confounded our findings. The absence of significant protection of the infant in association with maternal NFV therapy in this study might be due to suboptimal timing of NFV use, since 15% of women in the NFV-exposed group only began receiving NFV during the third trimester and 15% stopped treatment before delivery. CMV transplacental transmission rates from maternal primary infection increase with advancing gestational age from approximately 20% in the first trimester, to approximately 75% in the third trimester.(22, 23) Multivariable models were constructed to adjust for potential confounders but had limited power given the relatively small number of cCMV infections. Thus, we cannot rule out that NFV may have a modest effect on the risk of cCMV infection, especially if given throughout pregnancy at optimal doses. However, determining this would require a large randomized clinical trial.

Notably, the prevalence of cCMV infection found in our study (2.2%) – although high compared to the general population(1) – was in the lower range when compared to previously published rates of congenital CMV (range 1% – 10%) in studies of HIV-infected women,(2–8) including a French cohort study during the same time period that reported a prevalence of cCMV infection of 3 – 4%.(5) The total rate of cCMV infection in this study was likely underestimated by using blood, especially PBMC, to test for CMV, rather than saliva or urine samples. Numerous studies have reported that PCR of dried blood spots, serum, or whole blood had low sensitivity compared with urine or saliva rapid culture or PCR.(24–28) Although CMV can readily be detected in PBMC of viremic transplant recipients by PCR,(29–31) our significantly lower rate of cCMV among infants with PBMC compared to plasma suggest that PBMC may be inferior to detection of cCMV infection. The type of samples tested did not influence the effect of nelfinavir on cCMV infection that

was measured, based on the relatively equal numbers of PBMC and plasma samples in each group and because sample type was adjusted for in the multivariable analysis.

Risk factors for cCMV infection in HIV-exposed uninfected children have been previously described. Maternal CMV serostatus was not available from these cohorts; however, most HIV-1-infected pregnant women are co-infected with CMV even in resource-rich countries,(32) and maternal serostatus is a poor predictor of the risk of congenital CMV transmission.(33) Our findings are consistent with reports that type of maternal antiretroviral therapy is not associated with the rate of cCMV infection,(5, 7) whereas maternal CD4 T cell count <200 cells/mm^3 is independently associated with cCMV infection.(5) Similarly, earlier initiation of antiretrovirals in pregnancy appeared to be protective against cCMV infection risk in our cohort, supporting the findings of Guibert et al. that beginning antiretroviral treatment before or during the first trimester of pregnancy decreases the likelihood of infection compared to initiation during the second trimester,(5, 34) presumably due to improved immunologic control of maternal CMV replication.

cCMV infection has been associated with preterm delivery in both HIV-infected and uninfected women.(35, 36) Although the mechanism is unclear, placental infection with CMV often results in chronic villitis(37) and local cytokine modulation(38, 39) that is thought to cause preterm labor. Infants with cCMV infection were born at slightly earlier gestational ages compared to uninfected infants in this cohort, but there was no significant association with preterm birth.

Despite previous findings of *in vitro* activity against CMV replication,(11) in this study NFV did not protect HIV-exposed uninfected infants from cCMV infection. Other strategies to reduce congenital CMV infection, including valacyclovir(8) and passive immunization during pregnancy,(10) also did not appear to prevent cCMV infection. Given the profound morbidity that can result from cCMV infection, these failures highlight the need to better understand CMV transmission to the fetus, as well as for additional trials of pharmacologic interventions and maternal CMV vaccines. The high incidence of cCMV infection in infants of HIV-infected women argues for studies to determine if other antiretrovirals may provide dual protection by targeting sites shared by HIV and CMV.(40)

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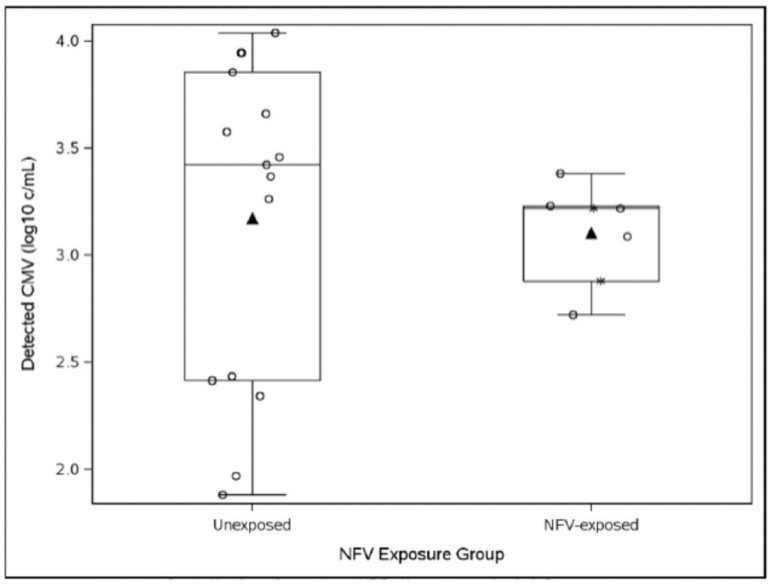

Figure 1. CMV DNA plasma viral load by nelfinavir exposure groups among CMV congenitally infected newborns (N=22)

The distribution of CMV DNA levels in plasma is not different between congenitally-infected infants (open-circles) whose mothers did or did not receive NFV during pregnancy. Box plots show the medians and interquartile ranges of the CMV DNA copies/ml in plasma for NFV-unexposed (n=15; median 3.42; IQR 2.41, 3.85) and NFV-exposed infants (n=7; median 3.22; IQR 2.88, 3.23; p=0.290); means shown by triangles. Asterisks indicate the values of two NFV-exposed infants whose mothers were no longer receiving NFV at the time of delivery.

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Table 1

Maternal, obstetrical and neonatal characteristics by nelfinavir exposure

			Nelfinavir exposure group		
Characteristics	**Total (N=1255)**	**Missing data**	**Unexposed (N=941)**	**Exposed (N=314)**	**P value***
cCMV status of infant					
CMV PCR positive	27 (2%)	0	19 (2%)	8 (3%)	0.653
Maternal characteristics					
Maternal age at time of delivery (IQR)	27.4 (23.1, 32.2)	0	27.5 (23.0, 32.2)	27.3 (23.8, 32.3)	0.848
Race/ethnicity		8			0.175
Non-Hispanic Black	734 (59%)		542 (58%)	192 (62%)	
Hispanic	354 (28%)		278 (30%)	76 (24%)	
Non-Hispanic White/Other	159 (13%)		115 (12%)	44 (14%)	
CDC HIV Classification		0			0.099
A	833 (80%)		675 (81%)	158 (75%)	
B	93 (9%)		68 (8%)	25 (12%)	
C	119 (11%)		90 (11%)	29 (14%)	
Study		0			
PACTG 316	661 (53%)		447 (48%)	214 (68%)	<0.001
P1025	594 (47%)		494 (52%)	100 (32%)	
Maternal HIV infection					
Timing of HIV diagnosis		2			
>2 years prior to pregnancy	597 (48%)		467 (50%)	130 (41%)	0.018
<2 years prior to pregnancy	233 (19%)		174 (19%)	59 (19%)	
During pregnancy	423 (34%)		298 (32%)	125 (40%)	
1st CD4 count <200 cell/uL during pregnancy	164 (13%)	19	112 (12%)	52 (17%)	0.043
Near delivery CD4 count <200 cells/uL	114 (11%)	235	74 (10%)	40 (15%)	0.006
1st HIV RNA during pregnancy ≤400c/mL	489 (45%)	171	355 (43%)	134 (50%)	0.170
Near delivery HIV RNA ≤400c/mL	725 (66%)	156	545 (67%)	180 (64%)	0.482
Antiretroviral exposure					
GA at 1st ARV use during pregnancy, weeks	14.4 (0.0, 21.0)	0	14.0 (0.0, 20.5)	16.1 (0.0, 22.9)	0.007
ARV, used for longest duration in pregnancy		0			<0.001

			Nelfinavir exposure group		
Characteristics	**Total (N=1255)**	**Missing data**	**Unexposed (N=941)**	**Exposed (N=314)**	**P value** *
No ARV	5 (0%)		5 (1%)	0 (0%)	
Monotherapy	160 (13%)		149 (16%)	11 (4%)	
Other	244 (19%)		225 (24%)	19 (6%)	
3 NRTIs	48 (4%)		46 (5%)	2 (1%)	
ART (≥3 drugs, ≥2 classes)	798 (64%)		516 (55%)	282 (90%)	
Total duration of ARV during pregnancy (weeks)	24.1 (17.7, 37.1)	0	24.3 (18.1, 37.1)	23.4 (17.1, 37.0)	0.531
Obstetrical characteristics					
GA at delivery, weeks	38.6 (37.7, 39.7)	0	38.6 (37.7, 39.6)	38.7 (38.0, 39.7)	0.207
Mode of delivery		6			
Vaginal delivery	679 (54%)		510 (54%)	169 (54%)	0.886
Cesarean after labor/membrane rupture	238 (19%)		175 (19%)	63 (20%)	
Elective Cesarean section	332 (26%)		252 (27%)	80 (25%)	
Infant characteristics					
Birth weight (kg)	3.0 (2.7, 3.4)	0	3.0 (2.7, 3.4)	3.1 (2.8, 3.4)	0.675
Infant birth size category		4			
Small for GA	67 (5%)	0	50 (5%)	17 (5%)	0.840
Appropriate for GA	1,108 (89%)		830 (88%)	278 (89%)	
Large for GA	74 (6%)		57 (6%)	17 (5%)	
Intrauterine growth retardation	2 (0%)		2 (0%)	0 (0%)	
Infant's age at specimen collection, weeks	1.1 (0.1, 2.1)	0	1.3 (0.1, 2.1)	0.9 (0.1, 2.0)	0.003
Specimen type: PBMC (vs. plasma)	491 (39%)	0	374 (40%)	117 (37%)	0.435

Data are presented as absolute frequency (%) or median (interquartile range).

*Wilcoxon Test was used to compare medians and Chi-Square Test was used to compare frequencies.

ARV: antiretroviral

ART: combination antiretroviral therapy using ≥3 drugs from ≥2 classes

GA: gestational age

NRTI: nucleoside reverse transcriptase inhibitor

c/mL: copies per milliliter

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Table 2

Maternal and infant characteristics by infant cCMV DNA PCR results from 0 to ≤3 weeks of age

Characteristics	Negative CMV PCR (N=1228)	Positive CMV PCR (N=27)	P-Value*
Demographics			
Maternal age at time of delivery	27.4 (23.1, 32.2)	27.3 (22.4, 33.3)	0.924
Race/ethnicity			
Black	714 (58%)	20 (74%)	0.110
Hispanic	351 (29%)	3 (11%)	
White/Other	155 (13%)	4 (15%)	
Study			
PACTG 316	648 (53%)	13 (48%)	0.699
P1025	580 (47%)	14 (52%)	
Maternal HIV infection			
First CD4 count <200 cells/uL during pregnancy	155 (13%)	9 (33%)	0.006
CD4 count <200 cells/uL near delivery	107 (9%)	7 (26%)	0.016
First viral load during pregnancy <=400c/mL	477 (39%)	12 (44%)	0.681
Viral load near delivery <=400c/mL	710 (58%)	15 (56%)	0.828
Antiretroviral exposure			
GA at first use of any ARV, weeks	14.3 (0.0, 21.0)	17.86 (10.9, 26.7)	0.037
Any ARV initiation prior to pregnancy (vs during)	406 (33%)	5 (19%)	0.146
Longest ARV regimen used during pregnancy			
Non-ART or no ARV	447 (36%)	10 (37%)	1.000
ART (≥3 drugs, ≥2 classes)	781 (64%)	17 (63%)	
Weeks of any ARV use during pregnancy	24.1 (17.7, 37.1)	21.9 (13.0, 35.4)	0.120
NFV-exposed (versus unexposed)	306 (25%)	8 (30%)	0.650
Weeks of NFV use during pregnancy	19.00 (12.6, 26.9)	19.50 (12.9, 24.4)	0.657
GA in weeks at first NFV use	17.86 (7.00, 24.29)	18.36 (8.43, 25.21)	0.789
Obstetrical characteristics			
Mode of delivery			0.465
Vaginal delivery	666 (54%)	13 (48%)	
Cesarean section after labor/membrane rupture	230 (19%)	8 (30%)	

Characteristics	Negative CMV PCR (N=1228)	Positive CMV PCR (N=27)	P-Value*
Elective Cesarean section	326 (27%)	6 (22%)	
GA in weeks at delivery	38.7 (37.7, 39.7)	38.0 (36.9, 38.7)	0.010
Preterm delivery (<37 weeks)	161 (13%)	7 (4%)	0.078
Infant characteristics			
Birth weight, kg	3.04 (2.74, 3.40)	2.87 (2.41, 3.12)	0.009
Small for GA/IUGR	68 (6%)	1 (4%)	1.000
Infant sex: male	615 (50%)	13 (48%)	0.846
Infant age at specimen collection, weeks	1.1 (0.1, 2.1)	1.71 (0.3, 2.3)	0.298
Specimen type: PBMC (versus plasma)	486 (40%)	5 (19%)	0.028

Data are presented as absolute frequency (%) or median (25th, 75th centile).

*Wilcoxon Test was used to compare medians and Fisher's exact Test was used to compare frequencies.

ARV: antiretroviral

ART: combination antiretroviral therapy using ≥3 drugs from ≥2 classes

GA: gestational age

NFV : nelfinavir

PBMC: peripheral blood mononuclear cells

cCMV – congenital cytomegalovirus infection

IUGR – intrauterine growth retardation

Table 3

Association of NFV exposure on cCMV infection in infants adjusted for potential confounders

	Unadjusted*		Adjusted*			
			Model 1		Model 2	
Covariates	**OR (95% CI)**	**P value**	**OR (95%CI)**	**P value**	**OR (95%CI)**	**P value**
NFV exposure (versus unexposed)	1.27 (0.48, 3.07)	0.710	1.20 (0.45, 2.92)	0.981	1.10 (0.41, 2.68)	0.980
First maternal CD4 count (<200 versus ≥200)	3.40 (1.32, 8.13)	0.011			3.56 (1.37, 8.61)	0.009
Specimen type PBMC vs. plasma	0.35 (0.10, 0.95)	0.036	0.36 (0.11, 0.99)	0.046		
ARV initiation prior to pregnancy vs. during	2.19 (0.80, 7.44)	0.153	2.07 (0.76, 7.08)	0.192	2.34 (0.85, 8.02)	0.116

*Exact logistic regression analysis. Each multivariable models include adjustment for all the covariates for which odds ratios and P values are shown (Model 1 includes NFV exposure, specimen type and ARV initiation prior to pregnancy; Model 2: includes NFV exposure, first maternal CD4 count, and ARV initiation prior to pregnancy).

OR (95% CI): odds ratio (95% confidence interval)

ARV: antiretroviral