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MAJOR ARTICLE

Cytomegalovirus Urinary Shedding in HIV-infected Pregnant Women and Congenital Cytomegalovirus Infection

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Background. Cytomegalovirus (CMV) urinary shedding in pregnant women infected with human immunodeficiency virus (HIV) was evaluated to determine whether it poses an increased risk for congenital CMV infection (cCMV).

Methods. A subset of mother-infant pairs enrolled in the perinatal NICHD HPTN 040 study (distinguished by no antiretroviral use before labor) was evaluated. Maternal and infant urines were tested by qualitative real-time polymerase chain reaction (RT-PCR) for CMV DNA with quantitative RT-PCR performed on positive specimens.

Results. Urine specimens were available for 260 women with 85.4% from the Americas and 14.6% from South Africa. Twenty-four women (9.2%) had detectable CMV viruria by qualitative PCR. Maternal CMV viruria was not associated with mean CD4 cell counts or HIV viral load but was associated with younger maternal age ($P = .02$). Overall, 10 of 260 infants (3.8%) had cCMV. Women with detectable peripartum CMV viruria were more likely to have infants with cCMV than those without: 20.8% (5/24) versus 2.1% (5/236), ($P = .0001$). Women with CMV viruria had significantly higher rates of HIV perinatal transmission (29.2% vs. 8.1%, $P = .002$). They were 5 times (adjusted odds ratio [aOR] = 5.6, 95% confidence interval [CI] 1.9–16.8) and nearly 30 times (aOR, 29.7; 95% CI, 5.4–164.2) more likely to transmit HIV and CMV to their infants, respectively. Maternal gonorrhea (aOR, 19.5; 95% CI, 2.5–151.3) and higher maternal HIV log10 viral load (OR, 2.8; 95% CI, 1.3–6.3) were also significant risk factors for cCMV.

Conclusion. In this cohort of HIV-infected pregnant women not on antiretrovirals, urinary CMV shedding was a significant risk factor for CMV and HIV transmission to infants.

Clinical Trials Registration Number. NCT00099359.

Keywords. HIV; pregnancy; congenital CMV; CMV viruria; HIV perinatal transmission.

Cytomegalovirus (CMV) is an important yet neglected cause of congenital infection, which may lead to sensorineural hearing loss and developmental delay [1, 2]. Congenital CMV infection (cCMV) may result from maternal primary infection, reactivation, or reinfection during pregnancy with CMV [3]. The prevalence of cCMV has been suggested to be ≤1% in industrialized nations but may be higher in resource-limited countries, where CMV seropositivity is higher [1, 4–6]. Other studies have suggested that cCMV rates may be higher among infants born to mothers infected with human immunodeficiency virus (HIV) in the pre-antiretroviral era compared to infants born to HIV-uninfected women [7–12]. HIV-infected infants may be more likely to demonstrate symptomatic cCMV than HIV-uninfected infants, and CMV infection may lead to more rapid progression of infant HIV infection [7–12].

CMV may be shed in bodily fluids such as saliva, breast milk, urine, and cervical secretions [3]. Studies evaluating CMV viruria in pregnant women have suggested intermittent detection

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Change of Author Affiliations: Lynne Mofenson, MD, has retired from service at the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, and is now Senior HIV Technical Advisor at the Elizabeth Glaser Pediatric AIDS Foundation, Washington, DC. D. Heather Watts, MD, was previously at the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, but is currently at the Office of the Global AIDS Coordinator, US Department of State, Washington, DC.

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of the virus in urine specimens [13–16]. Although detection of CMV in bodily fluids may be a marker of viral replication [17], the majority of prior studies in healthy pregnant women have not shown that maternal CMV shedding in urine and/or cervical secretions is associated with an increased risk of cCMV in infants [14, 16, 18–22].

However, less is known about the relevance of CMV shedding in urine and cervical specimens, among women with impaired cell-mediated immunity such as HIV-infected pregnant women [23]. This is of particular importance because impaired immunity may lead to reactivation or persistence of CMV infection [24].

In order to address the lack of published research evaluating the role of maternal CMV shedding and the development of congenital CMV in high-risk populations such as HIV-infected pregnant women, the present substudy of the NICHD HPTN 040 cohort was developed. The primary objective of this analysis was to evaluate rates of CMV viruria among HIV-infected pregnant women and to determine potential associations between maternal CMV viruria and cCMV. Additional secondary objectives include determination of risk factors for maternal CMV viruria, cCMV, and HIV perinatal transmission.

METHODS

The study population was a subset of mother-infant pairs enrolled in the National Institute of Child Health and Human Development (NICHD) HIV Prevention Trials Network (HPTN) 040 conducted from April 2004 to June 2011, for whom maternal and infant urines were available. One mL aliquots of maternal urine were tested with qualitative CMV DNA real-time polymerase chain reaction (RT-PCR), and positive specimens were tested by quantitative CMV DNA RT-PCR. Infant urines were similarly tested, with mother-infant pair results correlated.

Study Design

NICHD/HPTN 040 was a phase 3, triple-arm, randomized, open-label, multicenter study that evaluated the efficacy, safety, and tolerance of 3 different infant antiretroviral prophylaxis regimens for the prevention of intrapartum HIV transmission to infants born to HIV-infected pregnant women who had not received antiretroviral drugs during pregnancy [25].

HPTN 040 enrolled 1,684 HIV-infected pregnant women diagnosed with HIV infection at the time of labor and delivery from multiple sites in Brazil, South Africa, Argentina, and the United States. All women provided written informed consent prior to study enrollment. Infants <32 weeks of gestational age were excluded from study participation. Although the primary endpoint of the parent study was infant HIV infection status at 3 months of age, infants were followed until 6 months of age for safety and toxicity monitoring in the parent study.

At the time of labor and delivery, maternal plasma HIV RNA levels and CD4+ T-lymphocyte subsets were obtained. Serologic testing for syphilis was also performed at the time of labor and delivery per standard of care. Testing for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* was performed using the Xpert ® CT/NG assay (Cepheid, Sunnyvale, CA) on stored maternal urines.

HIV Diagnosis

Infant blood specimens were used to perform HIV DNA polymerase chain reaction (PCR) within 48 hours of birth and at 10–14 days, 4–6 weeks, 3 months, and 6 months of age. Positive infant HIV results were confirmed by repeat testing. Diagnosis of infant HIV infection required 2 positive HIV DNA PCR tests (Roche Molecular Systems Inc., Basel, Switzerland) collected on different days. In utero HIV infection was defined as a positive HIV DNA PCR test result at birth with positive results on repeat testing. *Intrapartum* HIV infection was defined as a negative HIV DNA PCR result at birth with a confirmed positive HIV DNA PCR result on subsequent testing. All HIV-exposed infants enrolled in the study were exclusively formula fed.

Specimen Collection and CMV Testing

Stored maternal urine samples, 1 per patient, were collected at the time of labor and delivery or within 48 hours after birth. Maternal urines were frozen at −80° C and stored at study sites. Thawed 1 mL aliquots of maternal urine were then tested by qualitative RT-PCR for CMV DNA (FOCUS Diagnostics CMV Analyte Specific Reagent). Maternal urines with positive qualitative results were then tested by quantitative CMV DNA RT-PCR. Infant urines also collected within 48 hours after birth were similarly tested with mother-infant pair results correlated. Infants with detectable CMV in urine in the first 48 hours of life were diagnosed with presumed cCMV. Testing of maternal and infant samples was performed after the conclusion of the parent study, and thus, results were not available to inform clinical management. Further detailed analysis of cCMV as a risk factor for HIV perinatal transmission as well as HIV perinatal transmission as a risk factor for cCMV are delineated in separate HPTN 040 substudies [26].

Statistical Analysis

The χ^2 (or Fisher exact test) was used to test the difference in proportions for categorical variables, and the 2-sample *t*-test (or Kruskal-Wallis) test was used to test the mean or median differences for continuous variables as appropriate. The univariate and multivariable logistic models were used to examine the associations of outcomes (infant HIV status, cCMV, and maternal CMV viruria) with potential risk factors, respectively. The

covariates with an overall Type III $P < .15$ from the univariate models were included in the initial full multivariable model for model selection. All computations were performed using SAS software v9.4 (Cary, NC, USA).

Human Subjects

The study was approved by the institutional review boards and national ethics committees at each participating site.

RESULTS

Maternal Profile

Urine specimens were available for 260 women. The majority of women (85.4%) were from the Americas (Brazil, Argentina, US); 14.6% were from South Africa. The mean maternal age was 27.1 years with the majority less than 30 years of age. High rates of bacterial sexually transmitted infections (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Treponema pallidum*), illegal substance use, alcohol use, and lack of prenatal care were observed (Table 1). The mean maternal CD4 count was 477 (SD 301) cells/mm^3, and the median maternal HIV viral load was 12 953 (range of 0–2 700 000) copies/mL.

Twenty-four women (9.2%) had detectable CMV viruria by qualitative PCR. The majority of women with viruria (91.7%) had low levels of detectable CMV (<200 copies/mL), whereas 2 women had higher levels at 236 and 53 524 CMV copies/mL, respectively. Of potential risk factors for maternal CMV viruria (infant antiretroviral regimen parent study arm, study region, prenatal care, sexually transmitted infections [STIs], alcohol use, tobacco use, maternal CD4 count, and maternal HIV viral load), only maternal age was a predictor for maternal CMV viruria in univariate analysis ($P = .02$). Mean age among women with CMV viruria was significantly lower (24.3 years, SD 4.8) compared to those without viruria (27.4 years, SD 6.5), $P = .006$. Younger women (ages 13–24 years and 25–29 years) were significantly more likely to have CMV viruria at the time of delivery (odds ratio [OR], 6.2; 95% confidence interval [CI], 1.4–28.3) and (OR, 5.4; 95% CI, 1.1–26), respectively. Mean HIV viral load and CD4 cell count were not significantly associated with CMV viruria ($P = .91$ and $P = .92$, respectively) (Table 1).

Maternal CMV Viruria and Other Risk Factors for Congenital CMV Infection

Overall, 10 (3.8%) infants had cCMV, with CMV detected from urine at the time of birth (range of 367–592 274 copies/mL). cCMV rates varied by detection of maternal CMV viruria. Among mothers with detectable urinary CMV at the time of delivery, 20.8% had infants with cCMV. In contrast, only 2.1% of infants born to mothers with undetectable urinary CMV had cCMV. The 2 women with the highest levels of CMV viruria had infants with cCMV. Women with CMV viruria were 12 times (OR, 12.2; 95% CI, 3.2–45.7) more likely to have an infant with cCMV. The relationship between maternal CMV viruria and cCMV was even more pronounced when the analysis was adjusted for mode of delivery, maternal gonococcal infection, and maternal HIV $\log_{10}$ viral load (aOR, 29.7; 95% CI, 5.4–164.2) (Table 2).

Apart from maternal CMV viruria, maternal gonococcal infection and maternal HIV viral load were also associated with cCMV. Women with gonococcal infection were six times more likely to have an infant with cCMV (OR, 6; 95% CI, 1.1–32.5), a difference that was even more pronounced in the adjusted analysis (aOR, 19.5; 95% CI, 2.5–151.3), when controlled for maternal urine CMV, mode of delivery, and $\log_{10}$ HIV viral load. Higher maternal HIV $\log_{10}$ viral load were also associated with nearly a 3-fold increased risk of cCMV (OR, 2.8; 95% CI, 1.3–6.3). (Table 2).

Maternal Peripartum CMV Viruria and HIV Perinatal Transmission and Infant Mortality

Women with CMV viruria also had significantly higher rates of HIV transmission to their infants (29.2% vs. 8.1%, $P = .002$) and were nearly 5 times more likely to transmit HIV to their infants (OR, 4.7; 95% CI, 1.7–12.8) on univariate analysis, with similar findings on multivariate analysis adjusted for infant study arm, maternal log10 viral load, and duration of ruptured membranes (aOR, 5.6; 95% CI, 1.9–16.8). (Table 2). Among the 7 HIV-infected infants born to women with CMV viruria, 5 were infected with HIV in utero and 2 were infected intrapartum. The one infant with both cCMV and HIV infection was infected with HIV in utero. Although there was an association between maternal CMV viuria and infant CMV acquisition ($P = .0001$, Table 2) and infant HIV acquisition ($P = .002$, Table 2), we did not observe an association between infant HIV and congenital CMV (cCMV) infection ($P = .66$, Table 3). Significant observed risk factors for maternal CMV viruria, cCMV, and HIV perinatal transmission in this study are summarized in Figure 1.

DISCUSSION

We evaluated the association between maternal CMV viruria and both cCMV and perinatal HIV transmission in a cohort of HIV-infected pregnant women, who were not receiving antenatal antiretroviral drugs, and found that infants born to women with CMV viruria at the time of labor and delivery were significantly more likely to have cCMV as well as HIV infection.

The profile of women and infant pairs in this smaller substudy was similar to that of our other published NICHD HPTN 040 analyses [27–29]. Among the population of late-presenting HIV-infected pregnant women included in this subanalysis, the majority of women were from the Americas (primarily Brazil) and had high rates of illegal substance usage, alcohol usage, lack of prenatal care, and other sexually transmitted infections (chlamydia, gonorrhea, and syphilis) [27–29].

Table 1. Maternal Characteristics Summary and Risk Factors for Maternal CMV Viruria

	Total (*N* = 260)	CMV Viruria Detected (*N* = 24)	CMV Viruria Not Detected (*N* = 236)	Unadjusted	
	n (col %)	n (row %)	n (row %)	OR (95% CI)	*P*-value
Study arm					
ZDV	83 (31.9)	8 (9.6)	75 (90.4)	1.00	
ZDV+NVP	87 (33.5)	7 (8.0)	80 (92.0)	0.82 (0.28– 2.37)	.71
ZDV+3TC+NFV	90 (34.6)	9 (10.0)	81 (90.0)	1.04 (0.38 – 2.84)	.94
Maternal age (years)					
13–24	99 (38.1)	13 (13.1)	86 (86.9)	6.20 (1.36 – 28.31)	.02
25–29	77 (29.6)	9 (11.7)	68 (88.3)	5.43 (1.13 – 25.97)	.03
30 and older	84 (32.3)	2 (2.4)	82 (97.6)	1.00	
Mean maternal HIV viral load (SD) (copies/mL)	73 108 (249 313)	70 907 (253 893)	94 664 (202 185)	…	.91
Maternal HIV viral load, categorical (copies/mL)					
≤400	15 (5.8)	1 (6.7)	14 (93.3)	1.00	
401 to ≤ 10,000	101 (38.8)	9 (8.9)	92 (91.1)	1.37 (0.16 – 11.65)	.77
10,001 to 100,000	109 (41.9)	10 (9.2)	99 (90.8)	1.41 (0.17 – 11.91)	.75
Log10 of maternal HIV viral load	259 (99.6)	24 (9.3)	235 (90.7)	1.13 (0.70 – 1.83)	.62
Mean maternal CD4 count (SD) (cells/mm3)	477 (301)	442 (179)	481 (311)	…	.92
Maternal CD4 count (cells/mm3)/100	256 (98.5)	24 (9.4)	232 (90.6)	0.95 (0.82 – 1.11)	.53
Region					
Americas	222 (85.4)	23 (10.4)	199 (89.6)	1.00	
South Africa	38 (14.6)	1 (2.6)	37 (97.4)	0.23 (0.03 – 1.79)	.16
Syphilis					
Yes	20 (7.7)	1 (5.0)	19 (95.0)	0.49 (0.06 – 3.86)	.50
No	239 (92.3)	23 (9.6)	216 (90.4)	1.00	
CT or NG					
No	209 (82.9)	21 (10.0)	188 (90.0)	1.00	
Yes	43 (17.1)	2 (4.7)	41 (95.3)	0.44 (0.10–1.94)	.28
CT					
No	212 (84.1)	21 (9.9)	191 (90.1)	1.00	
Yes	40 (15.9)	2 (5.0)	38 (95.0)	0.48 (0.11–2.13)	.33
NG					
No	239 (94.8)	23 (9.6)	216 (90.4)	1.00	
Yes	13 (5.2)	0 (0.0)	13 (100)	0.54 (0–2.60)	.56
Any of these STIs					
Yes	59 (22.8)	3 (5.1)	56 (94.9)	0.46 (0.13–1.59)	.22
No	200 (77.2)	21 (10.5)	179 (89.5)	1.00	
Prenatal care					
No	99 (38.1)	8 (8.1)	91 (91.9)	1.00	
Yes	159 (61.2)	16 (10.1)	143 (89.9)	1.27 (0.52–3.09)	.59
Alcohol use during pregnancy					
≥1/week	25 (9.7)	2 (8.0)	23 (92.0)	1.18 (0.25–5.66)	.84
>1/month, <1/week	14 (5.4)	2 (14.3)	12 (85.7)	2.26 (0.45–11.38)	.32
≤1/month	60 (23.2)	9 (15.0)	51 (85.0)	2.39 (0.94– 6.10)	.07
Never	160 (61.8)	11 (6.9)	149 (93.1)	1.00	
Illegal substance use					
Yes	38 (14.7)	3 (7.9)	35 (92.1)	0.82 (0.23–2.88)	.75
No	221 (85.3)	21 (9.5)	200 (90.5)	1.00	

Abbreviations: 3TC = lamivudine; CI, confidence interval; CMV, cytomegalovirus; CT = *Chlamydia trachomatis*; HIV, human immunodeficiency virus; NFV = nelfinavir; NG = *Neisseria gonorrhoeae*; NVP = nevirapine; OR, odds ratio; SD, standard deviation; STI = sexually transmitted infection; ZDV = zidovudine.

The frequency of maternal CMV viruria (9.2%) detected at the time of labor and delivery corresponds to rates reported in prior studies of healthy pregnant and nonpregnant women without HIV, which have ranged from 1.4–13% [14, 17, 20, 22, 30]. A few isolated published studies have reported on the detection of CMV cervical shedding in HIV-infected pregnant women, among whom CMV shedding rates were extremely high (66%) [31]. Only 1 study has evaluated rates of CMV urinary shedding

Table 2. Unadjusted and Adjusted Risk Factors for Congenital CMV (cCMV) and Perinatal HIV Transmission

	CMV Positive (*N* = 10)	CMV Negative (*N* = 250)	Unadjusted		Adjusted		HIV Positive (*N* = 26)	HIV Negative (*N* =234)	Unadjusted		Adjusted	
	n (row %)	n (row %)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	n (row %)	n (row%)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Maternal CMV												
Detected	5 (20.8)	19 (79.2)	12.16 (3.23 - 45.74)	.0002	29.72 (5.38–164.15)	.0001	7 (29.2)	17 (70.8)	4.70 (1.73–12.75)	.002	5.57 (1.85–16.78)	.002
Not detected	5 (2.1)	231 (97.9)	1.00		1.00		19 (8.1)	217 (91.9)	1.00		1.00	
Study arm												
ZDV	2 (2.4)	81 (97.6)	1.00				14 (16.9)	69 (83.1)	1.00		1.00	
ZDV+NVP	3 (3.4)	84 (96.6)	1.45 (0.24–8.88)	.69			10 (11.5)	77 (88.5)	0.64 (0.27–1.53)	.32	0.65 (0.26–1.63)	.36
ZDV+3TC+NFV	5 (5.6)	85 (94.4)	2.38 (0.45 – 12.63)	.31			2 (2.2)	88 (97.8)	0.11 (0.02–0.51)	.01	0.10 (0.02–0.46)	.003
Log10 of HIV maternal viral load	10 (3.9)	249 (96.1)	2.82 (1.27 – 6.26)	.01			26 (10.0)	233 (90.0)	1.66 (1.03–2.69)	.04	1.72 (1.02–2.90)	.04
NG												
No	7 (2.9)	232 (97.1)	1.00		1.00		25 (10.5)	214 (89.5)	1.00			
Yes	2 (15.4)	11 (84.6)	6.03 (1.12 – 32.46)	.04	19.45 (2.50 – 151.34)	.005	1 (7.7)	12 (92.3)	0.71 (0.09–5.72)	.75		
Duration of rupture of membranes												
Unknown							3 (14.3)	18 (85.7)	2.57 (0.64–10.40)	.18		
>24 hours							2 (40.0)	3 (60.0)	10.30 (1.52–69.67)	.02		
12–24 hours							0 (0.0)	9 (100)	<0.01 (<0.01 to >999)	.98		
6 to <12 hours							3 (14.3)	18 (85.7)	2.57 (0.64–10.40)	.18		
5 to <6 hours							9 (16.1)	47 (83.9)	2.96 (1.11–7.89)	.03		
<0.5 hours							9 (6.1)	139 (93.9)	1.00			

Log_{10} of HIV maternal viral load indicates that for each 1 unit log10 viral load increase, the odds of having an infant with cCMV or HIV increase 2.8 times or 1.7 times, respectively.

Abbreviations: 3TC, lamivudine; CI, confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; NFV, nelfinavir; NG, *Neisseria gonorrhoeae*; NVP, nevirapine; OR, odds ratio; ZDV, zidovudine.

Table 3. HIV Perinatal Transmission and Congenital CMV (cCMV)

		Infant Congenital CMV (cCMV)		
	Total (*N* = 260)	CMV Negative (*N* = 250)	CMV Positive (*N* = 10)	
	n (%)	n (%)	n (%)	*P*-value
Infant HIV				
HIV negative	234 (90.0)	225 (90.0)	9 (90.0)	.66
HIV infected in utero	14 (5.4)	13 (5.2)	1 (10.0)	
HIV infected *intrapartum*	12 (4.6)	12 (4.8)	0 (0.0)	

Abbreviations: CMV, cytomegalovirus; HIV, human immunodeficiency virus.

in HIV-infected pregnant or nonpregnant women, reporting a prevalence of 7%, similar to our results [23]. Our study is distinguished from the earlier one from the late 1990s of HIV-infected pregnant women because it employed sensitive diagnostic methods to detect CMV viruria (using CMV PCR as opposed to culture) [32, 33] and its inclusion of high-risk HIV-infected pregnant women from countries outside the United States (primarily Brazil and South Africa), where seroprevalence of CMV and rates of cCMV are believed to be higher [23].

Nearly 21% of our HIV-infected pregnant women with CMV viruria at the time of labor and delivery had an infant with cCMV. These women had a nearly 30-fold (adjusted OR, 29.7)

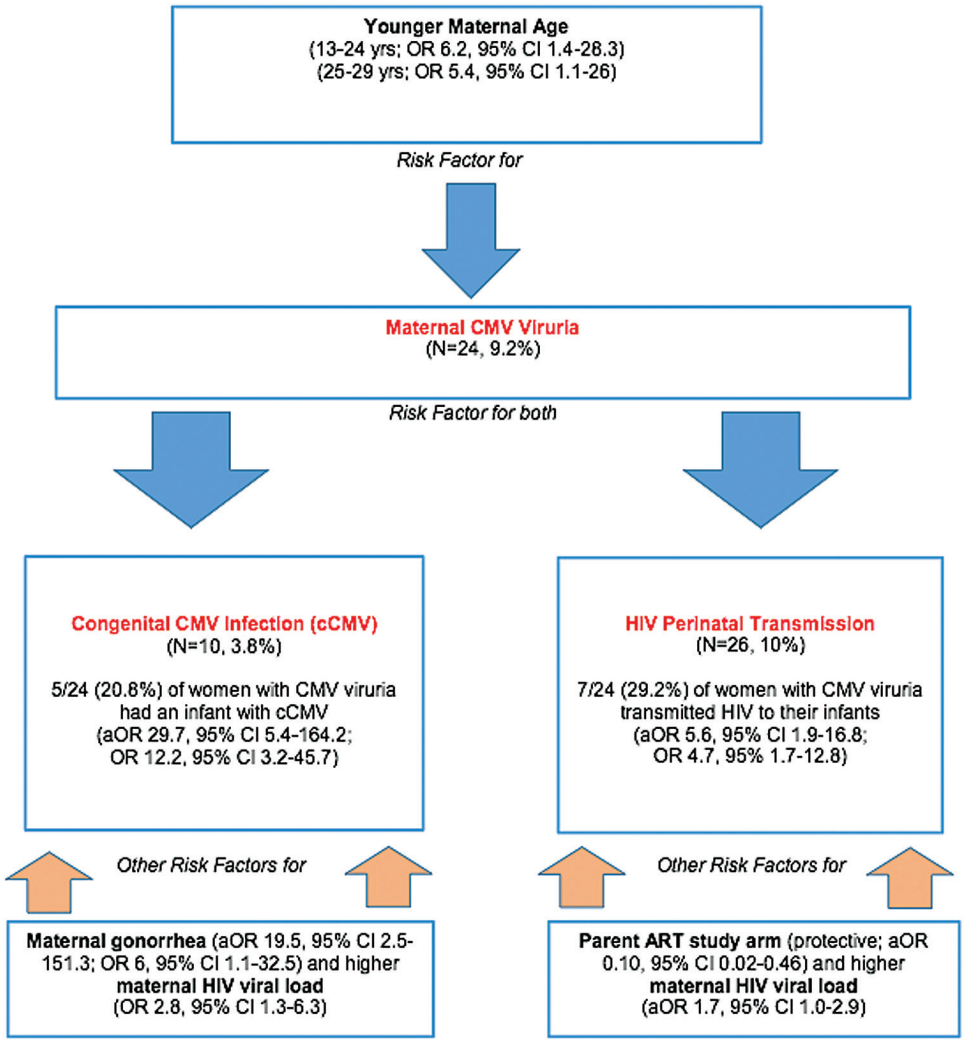

Figure 1. Summary of observed study risk factors for maternal CMV viruria, congenital CMV infection (cCMV), and HIV perinatal transmission. Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; OR, odds ratio.

increased risk of having an infant with cCMV as compared to women without CMV viruria. These findings contrast to the majority of other published studies, which have not found an association between maternal CMV urinary or cervical shedding and an increased risk of in utero CMV transmission [16, 18–22, 34, 35]. We are aware of only 1 other study that has documented high rates of cCMV among infants (10%) born to women with CMV viruria, which reported that high titers of maternal urinary CMV were significantly associated with cCMV [14]. Interestingly, that study, which took place more than 2 decades ago, only evaluated pregnant women without HIV infection. It is possible that the HIV infection status of pregnant women in our study, compared to the majority of prior studies, could account for the differing results.

Another interesting finding from our study was that maternal *Neisseria gonorrhoeae* was a risk factor for cCMV. Women with this STI were 6 times more likely (OR, 6; adjusted OR, 19.5) to have an infant with cCMV, and our findings correspond to results of other studies in the literature [3, 36–43].

Over 29% of women with CMV viruria transmitted HIV to their infants, and women with CMV viruria at the time of labor and delivery were 5 times (adjusted OR, 5.6) more likely to have an infant infected with HIV. These results contrast with the 1 other study evaluating maternal CMV viruria in HIV-infected pregnant women and its relationship to HIV perinatal transmission, which did not show a significant association of CMV viruria and transmission [23]. Our study also differed because it evaluated only HIV-infected pregnant women not receiving any antiretroviral drugs (such as zidovudine) during pregnancy.

Our findings that CMV viruria may be a marker for both in utero transmission of CMV *and* HIV underscores the complexities of these relationships [11, 12, 44]. One hypothesis for our findings is that immunosuppression inherent in pregnancy, particularly in the third trimester, along with that from HIV infection, may lead to more sustained local CMV reactivation and/or reinfection of the genital/urinary tract as evidenced by increased CMV viral shedding (i.e., viruria) [11, 12, 44]. In addition, CMV may also itself induce a pro-inflammatory state in the genital tract that may contribute to increased HIV-shedding [45]. These factors, along with decreased maternal antibodies and placental antibody transfer associated with untreated maternal HIV infection, may lead to an increased likelihood of CMV placental infection, inflammation, and ultimately CMV in utero transmission among infants as well as an increased risk of HIV perinatal infection, particularly in utero HIV transmission [11, 31].

One limitation of our study was that the sample size was restricted to women with available urine samples from our primary HPTN 040 study [25]. Thus, the lack of association between HIV perinatal transmission and cCMV was likely a reflection of our sample size. Furthermore, the magnitude of maternal CMV viruria detected in our cohort was low. The limited number of women with higher levels of CMV viruria (i.e., >200 copies/mL) in our study precluded determination of whether the degree of maternal viruria had any impact on the risk of CMV transmission to infants. Cervical specimens were also not collected in the parent study, which precluded evaluation of CMV cervical shedding rates. In addition, given the high seroprevalence of CMV among HIV-infected women in Brazil based on published studies by members of our team, maternal CMV serology was not done. We believe that the majority of maternal CMV infections in our cohort were likely due to CMV recurrence or reinfection [5, 6]. However, definitive information on maternal CMV seropositivity was not available for these women to make any conclusive statements in this regard. Women in our study were not on antiretroviral drugs during pregnancy; therefore, our findings should not be extrapolated to women who are adequately treated for HIV.

CONCLUSION

Urinary CMV shedding at the time of delivery in HIV-infected pregnant women not on antiretroviral drugs was relatively common in this high-risk cohort. Our findings suggest that maternal CMV viruria at the time of birth is a significant risk factor for both CMV and HIV transmission to infants born to women who did not receive antiretroviral treatment during pregnancy. In particular, our results appear to underscore the necessity of controlling maternal HIV infection during pregnancy through use of antiretrovirals to prevent both HIV and CMV transmission to neonates. Additional studies are needed to evaluate the role of both CMV urinary and genital shedding during pregnancy and the potential role this may play in the risk of in utero transmission of both HIV and CMV.

Notes

Author's contribution. K. A. drafted the initial substudy design and data analysis, drafted the initial manuscript, revised, and approved the final manuscript as submitted.

J. X. designed the data collection instruments, organized data entry for the initial study, and provided all data collected for this study. J. X. also assisted in providing methods for data analysis, confirmed and finalized primary data analysis study results presented in this paper.

B. A. assisted with preparation and coordination of urine samples from study sites and provided laboratory support in the United States.

E. J., J. H. P., B. S., R. F., R. K., J. P., and M. M. M.-P., G. G. and G. T. were responsible for initial study design, patient recruitment, and patient care enrollment in this study at sites in Brazil and in South Africa. They also reviewed and revised the manuscript and approved the final manuscript as submitted.

M. M. provided laboratory support in Brazil for study conduct, specimen storage, transfer of specimens to the United States, and participated in data analysis.

J. D. K. provided additional oversight for the current substudy design, data analysis, reviewed, revised, and approved final manuscript as submitted.

D. H. W., L. M., J. M., Y. B., V. V., and K. N.-S. supervised the original protocol development, design of the data collection instruments, supervised data collection at all sites, critically reviewed the manuscript, and approved of the final manuscript as submitted. K. N.-S. was the principal investigator of the parent study as well as this current substudy.

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