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Original Article

Prevalence and risk factors of cytomegalovirus infection among HIV-infected and HIV-exposed uninfected infants in Nigeria

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Abstract

Introduction: Cytomegalovirus (CMV) co-infection increases morbidity and mortality in human immunodeficiency virus (HIV) disease. There has been no study on CMV infection and its risk factors among Nigerian HIV-infected and/or HIV-exposed uninfected infants.

Methodology: This was a cross-sectional cohort study at the Federal Medical Center, Makurdi, between January 2012 and March 2013. Acute CMV infection among consecutive three-month- old HIV-infected and HIV-exposed uninfected infants was determined using the enzyme-linked immunosorbent assay of the CMV immunoglobulin M (IgM). The relationship between acute CMV infections in the infants and the potential risk factors was tested using logistic regression analyses.

Results: The prevalence of acute CMV infection was 41.4% (91/220), including 12.1% (11/91) and 87.9% (80/91) among the HIV-infected and the HIV-exposed uninfected infants, respectively. In multivariate logistic regression analyses, oropharyngeal candidiasis in the infants, HIV co-infection in the infants, maternal mastitis during breastfeeding, and the absence of maternal chronic CMV infections significantly increased the risk of acute CMV in the young infants.

Conclusions: In our setting, concerted efforts to prevent and/or promptly treat oropharyngeal candidiasis and mastitis during breastfeeding may reduce the burden of CMV among HIV-infected and HIV-exposed uninfected infants. Public enlightenment on the mode of CMV transmission and its prevention is also important.

Key words: cytomegalovirus; HIV; infants; prevalence; risk factors; Nigeria.

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Introduction

Cytomegalovirus (CMV) seroprevalence among women in the reproductive age ranges from 45% in developed countries to 100% in developing countries [1]. Maternal CMV can be transmitted to the infant *in utero*, perinatally, or postnatally [1]. *In utero* (congenital) infection can result from maternal primary infection, re-infection, or reactivation of latent infection [2]. Perinatal infection occurs from exposure to cervical and vaginal secretions during delivery [3]. Postnatal infection occurs after exposure to human milk, transplanted organs, or blood products [3]. Transfusion-associated cytomegalovirus infection (TA-CMV) is particularly noteworthy among the neonates and patients with advanced HIV disease. Unfortunately, CMV-seronegative blood products and third-generation filters that reduce the risk of TA-CMV are not readily available in resource-limited countries [4]. Furthermore, postnatal horizontal transmission of CMV to an infant through intrafamilial contacts with saliva or urine from an older child and/or the mother cannot be ignored in the context of pervasive poor personal and environmental hygiene and overcrowding that are common in resource-constrained countries [5]. CMV infection is important because it is the main infectious cause of mental retardation, neurodevelopmental impairment, and hearing loss in children, stemming from congenital infection *in utero* [6-9].

With an estimated 250,000 children infected with human immunodeficiency virus (HIV) in 2011, Nigeria accounts for more than 10% of the global pediatric HIV burden [10]. Complex interactions exist between HIV and CMV, including a shared mode of *in utero*, perinatal, and post-natal routes of transmission [11-14]; a higher rate of congenital CMV infection in HIV-infected infants [11-14]; an impaired containment of CMV replication among HIV-infected infants [15]; and a more severe course of HIV infection, accompanied by a higher rates of central nervous system complications [16]. Mortality is also higher in CMV-HIV co-infected infants [17], and poorer growth

and development had been recorded even among HIV-exposed uninfected (HEU) infants who were co-infected with CMV [18].

In Africa, few studies on CMV in pediatric populations have been done in The Gambia [5], Kenya [15], Zambia [18], and Ivory Coast [19], with none known to the researchers in Nigeria of HIV-infected or HEU children who are at risk of the aforementioned complex interactions.

The need for this study is also strengthened by the fact that the available data among pregnant women in Nigeria [20-23] have indicated that the prevalence of CMV infection is high, at between 54.3% and 97.2%, with an implication that Nigerian children may be at a high risk of acquiring CMV infection.

The site of the study is also instructive; Benue State, located in the north-central region of Nigeria, has consistently had the highest burden of HIV in Nigeria in 2005, 2008, and 2010 [24], implying that our findings may be generalized to reflect the burden of HIV-CMV co-infections among Nigerian children.

This study, therefore, aimed to determine the prevalence and the risk factors of acute CMV infections among HIV-infected and HEU infants in a pediatric antiretroviral therapy program in Makurdi, Nigeria.

Methodology

Study area and setting

This cross-sectional cohort study took place between January 2012 and March 2013 at the pediatric antiretroviral therapy (ART) clinic of the Riverside Specialist Clinics of the Federal Medical Center (FMC) in Makurdi. The center is the only tertiary health hospital providing care and treatment for children exposed to or infected with HIV in Benue State, Nigeria. It is also a referral center for primary and secondary health facilities in Benue State and the surrounding states of Taraba, Nasarawa, and Kogi. The facility is supported by the AIDS Prevention Initiative in Nigeria (APIN)/Harvard President's Emergency Plan for AIDS Relief (PEPFAE) program.

Consent and ethical approval

Ethical approval for the study was obtained from the research and ethics committee of the FMC, Makurdi. The study also complied with the Helsinki Declaration. Written consent of the mothers of the subjects was sought and obtained for the study. The study's objectives were explained to the mothers, and it was emphasized that the information obtained from the study would be treated with the utmost confidentiality and that anyone was at liberty to decline participation. It was also underscored that declination to participate would not affect the care and treatment of the subjects.

Study population

Included in the study were consecutive HIV-infected and HEU infants delivered to consenting HIV-infected mothers attending the pediatric ART clinic of FMC, Makurdi, within the duration of the study. Excluded were infants whose mothers declined to participate in the study.

Follow-up of HIV-positive pregnant mothers and their infants and operational definitions

Scheduled follow-up clinic attendance for HIV-exposed infants was every two weeks for the first six weeks, and every month for the first three months. In the programmatic protocol, early infant diagnoses with the DNA/PCR were offered at the sixth week and the third month of life. Universal co-trimoxazole prophylaxis was commenced for all infants from six weeks of age. Unscheduled visits also took place at any time when diagnostic assessments of presenting problems were done. Infants with a confirmed HIV infection were recruited into care and administered antiretroviral therapy. Those that were HIV negative at 3 months were followed up to 18 months of age before the discontinuation of care if they remained HIV negative. Prevention of mother-to-child transmission of HIV (PMTCT) interventions followed the 2010 World Health Organization (WHO) recommendations (option B) [25]. During the first antenatal care (ANC) visit, all pregnant women received HIV testing and counseling, and those found to be HIV infected were screened for clinical and immunological eligibility for the commencement of ART. Mothers who were eligible (*i.e.*, CD4 count $\leq$ 350 cells/mm^3) received a highly active antiretroviral therapy (HAART) regimen including zidovudine (AZT) plus lamivudine (3TC) plus nevirapine (NVP) from diagnosis, and the regimen was continued for life. Mothers that did not qualify for HAART (*i.e.*, CD4 count > 350 cells/mm^3) also received HAART from 14 weeks of gestation or at any time following diagnosis until one week after exposure to breast milk had ended. Prophylaxis for the HIV-exposed babies included daily doses of NVP, commenced within the first 72 hours of life and given for the first six weeks of life regardless of infant feeding options. The CD4 counts and HIV-1 ribonucleic acid (RNA) level of the mothers were measured at scheduled visits during ANC for FMC

Makurdi attendees. Pre-trained nurses provided the necessary information to enable mothers make informed decisions on either breastfeeding or replacement feeding. Exclusive breastfeeding (EBF) was defined as the infant receiving only breast milk from birth to six months of age from his/her mother and no other liquids or solids, with the exception of drops or syrups containing vitamins, mineral supplements, or drugs [26]. Replacement feeding (RF) was defined as provision of infant formula and the exclusion of all breastfeeding during the first six months of age [25]. Mixed breastfeeding was defined as giving breast milk with non-human milk or solids at any time during the first six months of age [26]. HIV-infected infants were defined as babies whose mothers were HIV-infected and who had had two positive consecutive DNA/PCR results at six weeks and three months of age. HIV-exposed babies were defined as babies whose mothers were HIV infected and who had had two negative consecutive DNA/PCR results at six weeks and three months of age. Acute or primary CMV infection in the infant identified when CMV immunoglobulin M (IgM) antibodies were positive at the third month of life [27,28]. For the infants, this may represent congenital, perinatal, and postnatal infection, as the CMV IgM antibodies may last for three to four months following a primary infection at these three time points [28]. The maternally derived CMV immunoglobulin G (IgG) against CMV infection persisted for between three and six months; thus, CMV immunoglobulin G (IgG) antibodies were not appropriate for the diagnosis of infants three months of age [27]. For the mothers, diagnosis of acute/primary CMV infection was considered when both CMV IgG and CMV IgM were positive [27,28]. Maternal chronic CMV infection was considered when only CMV IgG was positive [27,28].

Data collection and management

A study proforma was developed to capture relevant information about the subjects at their third month of life and their mothers, including family and social history that may increase the risk of perinatal CMV transmission. This information included the subjects' gender, birth weight, gestational age at birth, mode of delivery, history of neonatal admission, history of blood transfusion; the mothers' prolonged rupture of membrane of more than four hours, vaginal tear, and breast condition; oral thrush in the infants, and history of breastfeeding/mixed feeding/replacement feeding. The parity of the mothers, number of people living in the household, the mothers' level of education, marital status of the mothers, and the monthly income of the mothers (the Nigeria's minimum wage was 18,000 naira, an equivalent of 113 United States dollars). The last pre-delivery forms of mothers were retrieved to provide information on the CD4 count, the viral load, the hemoglobin level, and the status of hepatitis B surface antigen and hepatitis C antibodies.

Method of CMV IgM and IgG enzyme-linked immunosorbent assay (ELISA) assays

About 5 mL of venous blood were collected from the infant-mother pairs when the infants were three months old. The samples were analyzed at the (APIN)/Harvard PEPFAR laboratory of the FMC, Makurdi, using an ELISA test kit. The infants' samples were tested for CMV-specific immunoglobulin M (IgM). The mothers' samples were tested for both CMV IgG and CMV IgM. The procedure involved has been previously described [20-23]. The optical density (OD) was read using a microplate reader at 450 nm wavelength. Specimen OD ratio was calculated (specimen OD/calibrator OD). It was negative when OD ratio was ≤ 0.90 and positive when OD ratio was ≥ 0.90.

Statistical analyses

Descriptive statistics were tabulated as numbers and percentages for categorical variables. The main outcome variable in the analysis was the infants' acute CMV infection at the third month of life. Potential risk factors (including HIV status state) for acute CMV infection were tested for significance in bivariate logistic regression analyses. Risk factors that achieved a significance level of 0.1 were considered eligible for multivariate logistic regression analyses and were entered in one block. For all analyses, p values less than 0.05 were considered statistically significant. Statistical analysis was done using SPSS version 16.

Results

A total of 220 young infants were seen during the study period. This included 106 males and 114 females with a male-to-female ratio of 1:1.1. Among these infants, 15 were HIV infected, and 205 were HEU. The overall prevalence of acute CMV infection among the infants was 41.4% (91/220), including 12.1% (11/91) among the HIV-infected infants and 87.9% (80/91) among the HEU infants. The prevalence of chronic CMV infection among the HIV-infected mothers was 55.5% (122/220) and that of acute CMV infection was 12.3% (27/220).

As shown in Table 1 and Table 2, the multivariate logistic regression analyses revealed that oropharyngeal candidiasis and HIV infection in the infants as well as breast mastitis and chronic CMV infections in the mothers remained independently associated with acute CMV infection in the young infants at the third month of life.

The trend was such that the presence of oropharyngeal candidiasis in the infants increased the odds of acute CMV infection in the infant by 16 times (adjusted odds ratio [aOR]: 16.72; 95% confidence interval [CI]: 1.77–158.42; p = 0.014). Also, HIV infection was associated with a significant 12-fold increased risk of acute CMV infection in the infants (aOR; 12.64; 95% CI: 2.97–53.84; p = 0.001). In addition, when mothers had mastitis during breastfeeding, a significantly increased risk of acute CMV infection was found in the infants (aOR: 9.69; 95% CI: 1.58–59.50; p = 0.014). Furthermore, infants whose mothers did not have chronic CMV infection were also found to be at significant risk of acute CMV infection (aOR: 4.56; 95% CI: 1.87–11.09; p = 0.001).

Although low birth weight in the infant (< 2.5 kg), neonatal intensive care unit (NICU) admission, presence of jaundice and splenomegaly in the infants, and the mother's status of being without a partner were all significantly associated with acute CMV in the infants at bivariate analysis, none of these variables remained independently associated with acute CMV infection at multivariate analysis.

Discussion

In this cohort study, the prevalence of acute CMV infection was 41.4% (91/220), including 12.1% (11/91) and 87.9% (80/91) for the HIV-infected and the HEU infants, respectively. These values were lower than the prevalence of 93% among HIV-infected and the 90% among the HEU Kenyan infants at three months of age found in another study [15]. Also, the CMV prevalence of 41.4% in this study was higher than the respective 23.8% and 38.6% reported among Bulgarian [29] and Brazilian [30] hospitalized children of similar ages. In addition, the prevalence of acute CMV infection in this study was higher than the 15%–20% among HEU children [16] but lower than the 30%–40% among the HIV-infected children in the United States cohorts at six months of age [13]. The differences in the geographical locations and ethnicities, the varying socioeconomic conditions, differences in normative practices of breastfeeding, the diverse age at CMV diagnosis, the HIV status state, the different definitions ascribed to CMV diagnosis, and the differing risks of postnatal transmission of CMV that were found in our setting and those of others could explain the dissimilarities in the prevalence of CMV observed in our study and in those of others [2,3,13,15,16]. In this study, we defined acute CMV infection as infants who were CMV IgM positive at three months of age. Generally, in developing countries, the risk of postnatal acquisition of CMV infection had been noted to increase cumulatively with age, and to peak in the first years of life [27,31].

In our cohorts, HIV infection in the infants was found to significantly increase the risk of acute CMV infection in the infants. Kovacs *et al*. [16] had previously suggested that the increase in the co-infection rate could be caused by higher levels of cervical CMV shedding in HIV-infected women who also transmitted HIV to their infants at birth, as positive cervical CMV cultures had also been shown to correlate with perinatal CMV infection [32]. Also, because of impaired immunological surveillance, HIV-infected infants are unable to abort incipient CMV infections and are thus more susceptible to horizontal postnatal CMV acquisition [16,33]. However, maternal advanced HIV disease (*i.e.*, CD4 counts ≤ 200 cells/mm^3, viral load > 100,000 copies/mL), which could also explain a higher mother-to-child transmission of HIV and a higher shedding of more CMV in the saliva, cervix tissue, or urine, was not found to be significantly associated with CMV infection in the young infants in this study.

Our study also revealed that the presence of maternal mastitis during breastfeeding also increased the risk of perinatal CMV infection in the infant by nine times. Postnatal CMV transmission has been independently linked to breast milk CMV DNA load [34,35], and as in HIV transmission, the risk may be higher in infants whose mothers had mastitis during breastfeeding [36]. This postnatal CMV transmission may have occurred despite the fact that all the mothers of the subjects in our cohort were on HAART during pregnancy and throughout the breastfeeding period.

Table 1. Acute CMV infection in the infants and the infants' variables

Variables	Acute CMV infection in infants		Bivariate analysis			Multivariate analysis		
	No	Yes	cOR	95% CI	P value	aOR	95% CI	P value
Gender								
Female (Ref)	65 (50.4%)	49 (53.8%)	1.0					
Male	64 (49.6%)	42 (46.2%)	0.87	0.51–1.49	0.613	-	-	-
Tribe								
Tiv (Ref)	113 (87.6%)	77 (84.6%)	1.0					
Idoma	11 (8.5%)	12 (13.2%)	1.62	0.68–3.86	0.275	-	-	-
Others	5 (3.9%)	2 (2.2%)	0.59	0.11–3.15	0.541			
Gestational age at birth								
< 37 weeks	6 (4.7%)	3 (3.3%)	0.69	0.17–2.87	0. 619	-	-	-
≥ 37 weeks (Ref)	123 (95.3%)	88 (96.7%)	1.0					
Birth weight								
< 2.5kg	1 (0.8%)	22 (24.2%)	40.81	5.39–309.28	0.000	NA	NA	NA
≥ 2.5kg (Ref)	128 (99.2%)	69 (75.8%)	1.0					
Mode of delivery								
Emergent C/S	6 (4.6%)	2 (2.2%)	0.17	0.01–2.98	0.224	-	-	-
Vaginal	122 (94.6%)	87 (95.6%)	0.36	0.03–3.99	0.403	-	-	-
Elective C/S (Ref)	1 (0.8%)	2 (2.2%)	1.0					
Vaginal tear during delivery								
Yes	24 (18.6%)	25 (27.5%)	1.68	0.87–3.14	0.121	-	-	-
No (Ref)	105 (81.4%)	66 (72.5%)	1.0					
ROM before delivery								
< 4 hours	115 (89.1%)	87 (95.6%)	0.38	0.03–4.24	0.430	-	-	-
≥ 4 hours	13 (10.1%)	2 (2.2%)	0.08	0.00–1.29	0.075	0.05	0.00–3.48	0.170
Not applicable** (Ref)	1 (0.8%)	2 (2.2%)	1.0					
NICU admission								
Yes	2 (1.6%)	28 (30.8%)	28.22	6.52–122.25	0.000	6.06	0.39–92.58	0.195
No (Ref)	127 (98.4%)	63 (69.2%)	1.0					
Cumulative mode of infant feeding at 3 months								
Mixed feeding	8 (6.2%)	11 (12.1%)	1.96	0.75–5.13	0.170	-	-	-
EBF	24 (18.6%)	12 (13.2%)	0.71	0.33–1.52	0.383	-	-	-
RF (Ref)	97 (75.2%)	68 (74.7%)	1.0					

Table 1 *(continued)*. Acute CMV infection in the infants and the infants' variables

Variables	Acute CMV infection in infants		Bivariate analysis			Multivariate analysis		
	No	Yes	cOR	95% CI	P value	aOR	95% CI	P value
Oropharyngeal candidiasis								
Yes	1 (0.8%)	7 (7.7%)	10.67	1.29–88.27	0.028	16.72	1.76–158.42	0.014
No (Ref)	128 (99.2%)	84 (92.3%)	1.0					
Hepatomegaly								
Yes	11 (8.5%)	5 (5.5%)	0.62	0.21–1.86	0.397	-	-	-
No (Ref)	118 (91.5%)	86 (94.5%)	1.0					
Splenomegaly								
Yes	1 (0.8%)	9 (9.9%)	14.05	1.75–112.96	0.013	NA	NA	NA
No (Ref)	128 (99.2%)	82 (90.1%)	1.0					
Jaundice								
Yes	3 (2.3%)	14 (15.4%)	7.64	2.13–27.43	0.002	NA	NA	NA
No (Ref)	126 (97.7%)	77 (84.6%)	1.0					
Adenopathy								
Yes	6 (4.7%)	7 (7.7%)	1.71	0.55–5.26	0.351	-	-	-
No (Ref)	123 (95.3%)	84 (92.3%)	1.0					
Infants' HIV status state								
Positive	4 (3.1%)	11 (12.1%)	4.29	1.32–13.96	0.015	12.64	2.97–53.84	0.001
Negative (Ref)	125 (96.9%)	80 (87.9%)	1.0					
Blood transfusion in the infant*								
Yes	0 (0%)	12 (13.2%)	1					
No (Ref)	129 (100%)	79 (86.8%)	1.0					

* Omitted because of collinearity; ** Delivered via elective caesarean section; NA: not available; C/S: cesarean section; ROM: rupture of membrane; NICU: neonatal intensive care unit; EBF: exclusive breastfeeding; RF: replacement feeding; Ref: reference variable.

Table 2. Acute CMV infection in the infants and the mothers' variables

Variables	HCMV infection		Bivariate analysis			Multivariate analysis		
	No	Yes	cOR	95% CI	P value	aOR	95% CI	P value
Mothers' age groups								
< 25 years	13 (10.1%)	16 (17.6%)	1.90	0.26–27.38	0.409	-	-	-
≥ 25 years (Ref)	116 (89.9%)	75 (82.4%)	1.0					
Mothers' levels of education								
No formal	27 (21.0%)	24 (26.3%)	2.67	0.26–27.38	0.409	-	-	-
Primary	31 (24.0%)	28 (30.8%)	2.71	0.27–27.58	0.400	-	-	-
Secondary	68 (52.7%)	38 (41.8%)		0.17–16.68	0.659	-	-	-
Tertiary (Ref)	3 (2.3%)	1 (1.1%)	1.0					
Marital statuses of mothers								
Without partner	7 (5.4%)	15 (16.5%)	3.44	1.34–8.82	0.010	0.92	0.22–3.89	0.908
With partner (Ref)	122 (94.6%)	76 (83.5%)	1.0					
Mothers' monthly income*								
Below minimum wage	102 (79.1%)	70 (76.9%)	0.88	0.46–1.68	0.704	-	-	-
Above minimum age (Ref)	27 (20.9%)	21 (23.1%)	1.0					
Parity of the mothers								
> 1	78 (60.5%)	58 (63.7%)	1.15	0.66–2.00	0.623	-	-	-
1 (Ref)	51 (39.5%)	33 (36.3%)	1.0					
No. of people in household								
> 5	94 (72.9%)	72 (79.1%)	0.71	0.37–1.34	0.290	-	-	-
≤ 5 (Ref)	35 (27.1%)	19 (20.9%)	1.0					
Mothers' visits to kindergarten/crèche								
Yes	96 (74.4%)	70 (76.9%)	1.15	0.61–2.15	0.671	-	-	-
No (Ref)	33 (25.6%)	21 (23.1%)	1.0					
Mastitis during breastfeeding								
Yes	4(3.1%)	10 (11.0%)	3.86	1.17–12.72	0.027	9.69	1.58–59.50	0.014
No (Ref)	125 (96.9%)	81 (89.0%)	1.0					
Mothers' CD4 counts (cells/mm^3)								
≤ 200	5 (3.9%)	5 (5.5%)	1.44	0.41–5.13	0.572	-	-	-
> 200 (Ref)	124 (96.1%)	86 (94.5%)	1.0					

Table 2 *(continued)*. Acute CMV infection in the infants and the mothers' variables

Variables	HCMV infection		Bivariate analysis			Multivariate analysis		
	No	Yes	cOR	95% CI	P value	aOR	95% CI	P value
Mothers' viral loads (copies/mL)								
≤ 1,000 (Ref)	85 (65.9%)	52 (57.1%)	1.0					
1,001–10,000	22 (17.1%)	24 (26.4%)	1.78	0.91–3.49	0.092	0.92	0.29–2.94	0.883
10,001–100,000	17 (13.2%)	12 (13.2%)	1.15	0.51–2.61	0.731	-	-	-
> 100,000	5 (3.8%)	3 (3.3%)	0.98	0.22–4.28	0.979	-	-	-
Mothers' viral loads (copies/mL)								
Detectable	70 (54.3%)	53 (58.2%)	1.18	0.68–2.02	0.558	-	-	-
Undetectable (Ref)	59 (45.7%)	38 (41.8%)	1.0					
Mothers' anemia (Hb < 8 g/dL)								
Yes	22 (17.0%)	19 (20.9%)	1.28	0.65–2.54	0.474	-	-	-
No (Ref)	107 (83.0%)	72 (79.1%)	1.0					
Hepatitis B surface antigen								
Positive	20 (15.5%)	9 (9.9%)	0.59	0.26–1.38	0.229	-	-	-
Negative (Ref)	109 (84.5%)	82 (90.1%)	1.0					
Hepatitis C antibodies								
Positive	4 (3.1%)	5 (5.5%)	1.82	0.47–6.96	0.384	-	-	-
Negative (Ref)	125 (96.9%)	86 (94.5%)	1.0					
Mothers' CMV infections (chronic)								
Yes (Ref)	85 (65.9%)	37 (40.7%)	2.82	1.62–4.91	0.000	4.56	1.87–11.09	0.001
No	44 (34.1%)	54 (59.3%)	1.0					
Mothers' CMV infections (acute)**								
Yes	0 (0%)	27 (29.7%)	1					
No (Ref)	129 (100.0%)	64 (70.3%)	1.0					

*Nigeria's minimum wage is 18,000 naira (an equivalent of 113 United States dollars; ** Further analyses omitted because of collinearity; Hb: hemoglobin; Ref:reference variable

This also tends to support the study of Meyer *et al*. [37] in Malawi, whereby only minimal impact of HAART on breast milk CMV load was seen, suggesting that expanded maternal use of antiretroviral therapy may not reduce the risk of infant postnatal CMV acquisition via breast milk [37]. In the cohort of Gantt *et al.*'s study [38], mastitis was not seen in CMV-Epstein Barr virus co-infection, even though both viruses could cause or be reactivated by inflammation.

Not surprisingly, the presence of oropharyngeal candidiasis in the infants significantly increased the risk of CMV by 16 times. Again, in a similar fashion ascribed to HIV infection, CMV may infect the invading macrophages that are mobilized towards the breached mucosal surfaces and lesions in the mouths of infants during breastfeeding [39].

Furthermore, infants whose mothers did not have chronic CMV infections were four times more likely to have acute CMV infection in our cohort. Although we did not set out to assay maternally derived CMV IgG in our young infants, it is well known that transplacental-derived maternal CMV IgG antibody is protective against primary CMV infection in infancy [40]. Even when primary CMV infection still occurred despite maternal anti-CMV IgG as reported by Chen *et al*. [41], the infected children showed no symptoms or features related to CMV infection. In our study, the presence of jaundice and splenomegaly – features suggestive of CMV infection – were not significantly associated with acute CMV infection in the infants, which may support Chen *et al.*'s study [41].

In this study, all the 11 HIV-infected infants with acute CMV infection were promptly commenced on HAART. It is expected that HAART, when taken for three to six months, controls HIV replication and stimulates the immune system to control CMV infection [42]. All the 11 HIV-CMV co-infected infants also had a consultation with the ophthalmologist and none had retinitis.

Limitations of study

We were constrained to screening for CMV infection at the third month of life and not earlier because we wanted to make use of the infants' HIV status knowledge, which was only possible with the second DNA/PCR at the third month of life in our program protocol. Fortunately, CMV acquisition had been noted to peak in sub-Saharan HIV-exposed and HIV-infected infants at three to four months of age [15,44].

The CMV IgM positivity at the third month of life, however, could not clearly differentiate whether CMV infection was acquired congenitally, perinatally, or postnatally. Since cord blood CMV IgM was not assayed nor could urine CMV DNA be done at birth in our health facility, we could not totally rule out a possibility of congenital infection among subjects with a positive CMV IgM result.

Also, although a negative CMV IgM result suggests that an individual is not experiencing a recent infection, it may not totally rule out a reactivated CMV infection. The CMV IgG low avidity test, which could have differentiated clearly between acute CMV infection and CMV reactivation [45] was, unfortunately, not available in our health facility and in Nigeria.

Finally, because of constrained resources, we did not test for CMV infection among comparators of infants who were HIV uninfected and HIV unexposed.

Conclusions

In our cohort, a high prevalence of acute CMV infection (41.4%) was found. The presence of oropharyngeal candidiasis in the infants, HIV co-infection in the infants, absence of maternal chronic CMV infections, and maternal mastitis during breastfeeding were all independent risk factors of acute CMV. Therefore, concerted efforts to prevent and/or promptly treat oropharyngeal candidiasis in infants and maternal mastitis during breastfeeding may reduce the burden of acute CMV in HIV-infected and HEU infants in our setting. Also, concerted efforts aimed at reducing HIV and CMV infections in the general population cannot be overemphasized. The efficacy of a recombinant glycoprotein B CMV vaccine has been proven, and it may be useful in CMV infection control in Nigeria and in many other sub-Saharan African countries [43]. We also hope that the findings of this study will stimulate authors and other researchers in Nigeria to undertake longitudinal studies aiming to document the impact of CMV infection on HIV disease and vice versa.

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