Clinical Infectious Diseases Advance Access published November 21, 2013

High rates of congenital cytomegalovirus (CMV) infections linked with maternal HIV infection among neonatal admissions at a large referral centre in sub-Saharan Africa

Nyaxewo Mwaanza[1,2,*], Lophina Chilukutu[1,3,*], John Tembo[1,3,*], Mwila Kabwe[1], Kunda Musonda[4], Monica Kapasa[2], Chishala Chabala[1,2], Sylvester Sinyangwe[1,2], Peter Mwaba[1,5], Alimuddin Zumla[1,6], Matthew Bates[1,3,6,*]

[1]University of Zambia and University College London Medical School (UNZA-UCLMS) Research and Training Programme (www.unza-uclms.org), University Teaching Hospital, Lusaka, Zambia

[2]Department of Paediatrics and Child Health, University Teaching Hospital, Lusaka, Zambia

[3]HerpeZ (www.herpez.org), University Teaching Hospital, Lusaka, Zambia

[4]Department of Infectious Tropical Diseases, London School of Hygiene & Tropical Medicine, London, U.K

[5]Zambian Ministry of Health, Ndeke House, Lusaka, Zambia

[6]Department of Infection, Division of Infection and Immunity, University College London, London, UK

Correspondence: matthew.bates@ucl.ac.uk

[*]Authors contributed equally

Key Points: There is a growing awareness of the possible importance of congenital CMV infections in high seroprevalence populations. We show for the first time high rates of congenital CMV in neonatal admissions linked with maternal HIV infection in sub-Saharan Africa.

ABSTRACT

Background

Congenital cytomegalovirus (CMV) infection is the major infectious cause of birth defects and hearing loss globally. There is a growing recognition of the potential clinical impact of congenital CMV infections in high seroprevalence settings.

Methods

A cross-sectional study of neonatal admissions at a large referral centre in sub-Saharan Africa to determine the prevalence of both symptomatic and asymptomatic congenital CMV infection. Real Time PCR was used to screen DNA-extracted sera, urine and saliva, and an ELISA assay was used to screen sera for anti-CMV IgM. Multivariate binary logistic regression was used to identify risk factors associated with increased odds of congenital CMV infection.

Results

Congenital CMV was detected in 3.8% (15/395) of neonates. Among these cases 40% (6/15) presented with jaundice, one of which also had petechiae. Congenital CMV infection was detected in 11.4% (9/79) [6.1-20.3%] of neonates born to HIV-infected mothers and both maternal HIV (OR 6.661 [2.126-20.876], p = 0.001) and jaundice (OR 5.701 [1.776-18.306], p = 0.003) were independently linked with significantly increased odds of congenital CMV infection.

Conclusion

Congenital and early infant CMV infections may have important consequences for child health in sub-Saharan Africa and other high HIV and CMV seroprevalence populations globally.

INTRODUCTION

Congenital cytomegalovirus (CMV) infection occurs in 1% of births and is the leading infectious cause of birth defects globally [1]. The most damaging congenital CMV infections arise from maternal primary infections during pregnancy, which can result in mental retardation, and also account for 25% of all cases of hearing loss by 4 years of age [2]. Primary infection during pregnancy, and resultant severe congenital disease, is most common in industrialized countries where seroprevalence is lowest; with white ethnicity, higher socioeconomic status and lower parity being the most prominent risk factors [3, 4]. Within non-white women of the same ethnic group, seroprevalence was higher in those born in their country of ethnic origin, with family structure and arrangements for child-care likely playing an important role [4].

In sub-Saharan Africa it is well established that CMV seroprevalence is high among women of child-bearing age [5, 6] and so the perceived wisdom has been that the prevalence or clinical impact of congenital CMV must be low, resulting primarily from maternal re-infections or reactivations [7, 8]. There are three studies that actually evaluate the prevalence of congenital CMV infection in sub-Saharan Africa, giving rates of 1.4%-14% [9-11]. There were important methodological differences between these studies, but even at the lowest estimate, the prevalence of congenital CMV was comparable with that seen in Europe and North America [1]. With respect to outcomes in sub-Saharan Africa, an insufficient number of cases have been followed up, although from 54 cases, there have been two documented cases of severe neurological damage and one case of hearing loss [9]. One of the studies identified that lower parity, overcrowding and placental malaria were risk factors linked with congenital CMV infection [11]. There is a growing appreciation of the possible importance of congenital CMV infection in high seroprevalence populations [12], with large studies in Brazil confirming high rates of congenital CMV infection, linked with hearing loss [13, 14].

CMV infection is a defining opportunistic infection of AIDS progression in children [15], and there is increased prevalence of congenital CMV in HIV-infected versus HIV-uninfected children [16]. Congenital CMV was also more common in HIV negative children born to HIV-infected mothers not on ART, compared to those on ART [17]. This U.S data is of great relevance to sub-Saharan Africa, which is the epicentre of the HIV pandemic, yet the

prevalence and clinical impact of congenital and early childhood CMV infections in this region have been little studied.

In Zambia we have recently shown that CMV seroprevalence is high (83.5%) in healthy 18 month-old infants, and that these early infant CMV infections are linked with impaired physical development, and in maternally HIV-exposed children lower psychomotor development [18], although it was not possible to delineate the relative contributions of congenital and early infant CMV infections. In the absence of any baseline data on the prevalence of congenital CMV in Zambia, we chose to first investigate a high morbidity patient group in whom there might be a raised probability of symptomatic congenital CMV. In this study we evaluate the prevalence of congenital CMV infections, describe the clinical presentation and identify risk factors, among neonates admitted to the neonatal unit at the University Teaching Hospital, Lusaka, Zambia.

METHODS

Ethics approval

The study was approved by the Biomedical Research Ethics Committee of the University of Zambia School of Medicine (UNZABREC), Lusaka, Zambia. The mothers/guardians of all participants gave written informed consent.

Setting

The study was based on the neonatal unit of the University Teaching Hospital, Zambia's national referral centre. The unit has two for intensive care wards and also a recovery area, and so receives a number of relatively stable neonates in addition to those who require intensive care.

Study Design and Aims

The design was that of a cross-sectional observational study with the aim of determining the prevalence of congenital CMV infection, describing the clinical presentation and identifying associated risk factors. All neonatal admissions under 3 weeks of age were eligible for inclusion on to the study. We defined congenital CMV infection as detection of CMV DNA in any specimen (sera, saliva or urine), or CMV IgM in sera, in the first 3 weeks of life. CMV culture facilities were not available at the study site. Whilst detection of CMV IgM alone in neonates is not definitive proof of congenital CMV infection, it was included here for

research purposes, in addition to the three PCR assays, as there is very little data from our region on anti-CMV IgM detection in neonates.

Patient Recruitment

There were a total of 1806 neonatal admissions during the study period (11th Nov 2012 – 25th Apr 2013). Our recruitment team approached 1003 mothers during this period. Neonates admitted during the weekend or on bank holidays were not recruited due to limited available resources. Consent was obtained from 395 mothers. At least one specimen was collected from each patient (Figure 1). Patient recruitment, clinical evaluation, mother's medical/obstetric history and sample collection were undertaken by the study paediatrician and an UNZA-UCLMS research clinical officer.

DNA Extraction

Laboratory analysis was conducted in our dedicated 3-room molecular diagnostics laboratory. DNA from saliva and serum was extracted using the QIAamp DNA Mini Kit, and from urine using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's specifications. Extraction controls were included with every batch of 11 samples. DNA extraction quality was monitored on every 11th sample using a Nanodrop (Thermo Fisher Scientific, Waltham, MA).

PCR

Samples were tested for presence of CMV DNA by Real-time Taqman PCR assay, with a reported sensitivity and specificity 93.1% and 96.6% respectively [19]. Real-time PCR was carried out using a Rotor-Gene™ 6000 (Qiagen, Hilden, Germany). CMV genomic DNA from isolate AD169 (kindly provided by Ursula Gompels, London School of Hygiene & Tropical Medicine) was used as a positive control. The fidelity of the PCR enzyme and purity of the DNA-extraction was also controlled through amplification of the house-keeping gene, β-Actin (Table 1), from every 11th sample. Positive, negative (molecular grade water), reagent (no template) and extraction controls were included with each run. Oligonucleotide sequences and cycling conditions for both assays were as indicated (Table 1).

ELISA

Serum collected from the neonates was tested for the presence of anti-CMV IgM using the ETI-CYTOK-M reverse PLUS™ ELISA kit (Diasorin, Seluggia, Italy), according to manufacturer's

instructions. Positive, negative and cut-off controls were included in all runs and positive samples were retested to confirm initial positive result.

STATISTICAL ANALYSIS

Data analysis was undertaken using SPSS version 21 (IBM, Armonk, NY, USA). Pearson chi-squared was used to compare congenital CMV prevalence between different specimen types, and also for the comparison of gender, gestational age, maternal HIV status and neonatal mortality between the study group and the general neonatal inpatient population. Birth weight was compared using Mann Whitney U. Univariate and multivariate binary logistic regression was used to evaluate risk factors associated with congenital CMV infection.

RESULTS

Characteristics of the study population

Our aim was to recruit a representative sample from the inpatient neonatal population and so we collected gender, gestational age, birth weight, maternal HIV status and mortality data from all admissions during June-July 2013, and compared this with our study group. This analysis does not account for seasonal variation but is a rough indication of the degree to which our sample was representative of the population (Table 2). Mortality data for the population indicates some infants could not have been recruited onto the study, such as those who died shortly after admission and some who died en-route to the neonatal unit. This could explain the under-representation of both mortalities and pre-term neonates. Recruited neonates may also have received improved care. There was a trend for a selection bias towards male neonates possibly due to ease of urine collection. The prevalence of maternal HIV infection did not differ significantly between the study group (21.2%) and the general population (20.9%)(Table 2).

Detection and prevalence of congenital CMV infection

In the absence of facilities for culturing CMV, we chose a quadruple strategy defining congenital CMV infection as the detection of CMV DNA in saliva, urine or sera, or detection of IgM antibody in sera, in the first 3 weeks post-partum. This strategy helped compensate for the fact that congenital CMV infection does not always involve detectable virus shedding from any one site [20], with a previous study from Gambia showing a very poor correlation between CMV culture results from urine and saliva [9]. We detected congenital CMV

infection in 3.8% (15/395) of neonatal admissions (Table 3). The correlation between the four different screens was low, with only 3/15 cases being positive in more than one specimen. Urine gave the greatest yield (4.4%, 8/183), significantly greater than that of saliva (0.97%, 3/308, p = 0.017) or sera IgM (0.8%, 3/353, p = 0.01) but not significantly greater than that of sera PCR (1.7%, 6/355, p = 0.062). A quantification standard to calculate CMV viral load was not available but by analysing crude CT values, median viral loads were comparable between urine and sera, and possibly higher in saliva (Table 3).

Factors linked with congenital CMV infection

The prevalence of congenital CMV among neonates born to HIV-infected mothers was 11.4% (9/79) [95% CI 6.1-20.3%] compared to 2.1% (6/293) [95% CI 0.8-4.6%] in those born to HIV-uninfected mothers (Figure 2 & Table 4). Similarly, the prevalence of congenital CMV among neonates with jaundice was 10.5% (6/57) [95% CI 4.4-22.2%] compared to 2.5% (8/317) [95% CI 1.2-5.1%] in those without jaundice. Multivariate binary logistic regression confirmed that maternal HIV infection (OR 6.661 [95% CI 2.126-20.876], p = 0.001) and neonatal jaundice (OR 5.701 [95% CI 1.776-18.306], p = 0.003) were independently associated with significantly increased odds of congenital CMV infection (Table 4). These findings held when the two children in which anti-CMV IgM alone was detected, were coded as negative (Table 4, footnote). Just one neonate presented with petechiae, and this was also one of the 15 cases of congenital CMV, and hence petechiae was more prevalent among congenital CMV cases than among non congenital CMV cases: 0% vs 7.1% (p = 0.037)(Data not shown). From partial data we did not see any link between congenital CMV and haemoglobin level, platelet count, aspartate or alanine transaminase (data not shown).

Symptomatic congenital CMV infection and mortality

The mortality rate of our study group was 17.1%. Maternal HIV infection and pre-term birth were independently linked with mortality (data not shown), but congenital CMV infection was not linked with significantly increased odds of death (OR 1.818 [0.560-5.899], p = 0.319)(data not shown). 26.7% (4/15) of congenital CMV cases died, compared with 16.7% (61/366) of neonates without congenital CMV (Fishers exact p = 0.242). Six congenital CMV cases (40%) were admitted with a clinical presentation suggestive of symptomatic congenital CMV infection: Five cases of jaundice and one case of jaundice with petechiae. Two of the neonates with jaundice died. The first was a term baby admitted with respiratory distress syndrome born to an HIV negative mother. The second was a pre-term baby of 1.5kg,

admitted due to prematurity and grunting, born to an HIV-infected mother. The remaining 9 cases of congenital CMV which did not present with symptoms, were admitted for a range of reasons including prematurity, respiratory distress, birth asphyxia, suspected sepsis, meconium aspiration and macrosomia, none of which were linked with an increase in the odds of congenital CMV infection (Data not shown).

DISCUSSION

There are three key findings from this study: First, there was a high prevalence (3.8%) of congenital CMV on our neonatal unit; Second, symptoms associated with symptomatic congenital CMV were observed in up to 40% of cases, linked with mortality; and third, this is the first demonstration of a strong association between maternal HIV infection and congenital CMV infection in a high HIV and CMV seroprevalence population in sub-Saharan Africa.

In Zambia early infant CMV infections have been linked with impaired physical development and in HIV-exposed children, impaired psychomotor development [18], although the degree to which congenital transmission of CMV might have contributed to these effects was not determined. With no baseline data on the prevalence of congenital CMV in Zambia we chose to screen a high morbidity/high mortality inpatient group, to get the first indication of prevalence, to assess whether congenital CMV could cause symptomatic disease in this setting, and to identify risk factors linked with congenital CMV of possible use in the design of broader population-based studies.

We define congenital CMV as detection of viral DNA in infant saliva, urine or sera, or detection of anti-CMV IgM in infant sera. Detection of IgM alone is not considered definitive proof of CMV infection, with false positive results arising from cross-reactivity with other pathogens (regretfully we did not have sufficient resources to screen for other congenital and neonatal infections) and false negative results due to very young and/or immunocompromised neonates not mounting a detectable response to infection [21, 22]. For these reasons the true prevalence of congenital CMV infection in our study group is most accurately represented by our PCR results (up to 4.4% in urine). Whilst our study group were not representative of the population, a prevalence ranging from 1-5% is confluent with the findings of three previous population-based studies assessing congenital CMV prevalence in sub-Saharan Africa. The first was from the Ivory Coast and dates back to 1978,

where CMV was cultured from the urine of 1.4% (n = 2032) of healthy neonates in the first 12 hours post-partum [10]. 1.4% is a likely under-estimate of the true prevalence in accordance with the current accepted definition of congenital CMV, which is the detection of virus within 3 weeks post-partum. There are then two studies on the prevalence of congenital CMV from Gambia: the first in 1991 cultured CMV from either the urine or saliva from 14% of healthy neonates (n = 184), with a strong discordance between saliva and urine detection suggesting that screening just one specimen type might result in underestimates of prevalence [9]. Importantly, this study documented developmental defects and neurological damage in congenitally infected neonates. The second Gambian study detected CMV by PCR in the urine of 5.4% of healthy infants (n = 741) [11]. This study performed one year of follow-up, documenting more frequent health complaints in congenitally infected children but did not identify any neurological deficits.

The broad discordance between specimen type (urine vs saliva vs sera) and assay type (sera PCR vs sera IgM) in our study and previously [9] suggest that sites of viral shedding may vary, possibly influenced by time or frequency of maternal viral shedding during pregnancy, and that detection in one specimen alone should be considered a minimum estimate [20]. Future studies should screen multiple specimens until the long-term clinical impact of congenital CMV infections in sub-Saharan Africa is better understood. Our molecular analysis was undertaken in a dedicated molecular diagnostics suite, with rigorous controls, and we are confident that positive results indicate the detection of CMV DNA. To what degree they represent active infections that might be causing pathology requires larger longitudinal studies.

We observed symptoms possibly indicative of symptomatic congenital CMV infection in 40% (6/15) of cases, possibly higher than studies from industrialized countries where clearly defined symptomatic infection is seen in 10-15% of congenitally infected neonates [12] likely because we recruited admitted neonates. Two of the symptomatic cases died with a point mortality rate of 33% (2/6). Taking into account the gross under-representation of early neonatal deaths in our cohort, it is possible that we did not capture some additional congenital CMV-associated deaths, which may occur in 9% of symptomatic cases in low seroprevalence (CMV and HIV) settings [1]. This may also explain why we did not document other common symptoms such as neurological abnormalities, low birth-weight and hepatosplenomegally [17]. The degree to which our results may inform on prevalence

among healthy neonates cannot be determined, but even by the lowest estimates, our data and that from previous population-based studies in sub-Saharan Africa [9-11], suggest rates of congenital CMV infection at least as high as those seen in low-seroprevalence populations in industrialized countries, with isolated cases of neurological disease and hearing loss.

Studies from the U.S have demonstrated that CMV shedding in the genital tract of HIV-infected women, and in the cervical fluid and PBMCs of pregnant HIV-infected women, correlates strongly with reduced CD4 [23, 24] and raised HIV viral load [25]. Immune suppression in HIV-infected pregnant women likely leads to increased incidence of re-infection or reactivation, or prolonged CMV viral shedding, lengthening the opportunity for congenital transmission. Other U.S studies have shown higher prevalence [15, 16, 26] and poorer outcomes [15] due to congenital CMV infection in HIV-infected and/or exposed children and higher prevalence of congenital CMV in children born to HIV-infected mothers who have not initiated ART [17]. A study from Kenya detected CMV shedding in the genital tract of 59% of HIV-infected women [27], yet congenital CMV in the context of maternal HIV infection has not been previously studied in sub-Saharan Africa.

Here we show for the first time in the region, that maternal HIV infection is strongly associated with congenital CMV infection, which is detectable in up to 10% of children born to HIV-infected mothers. The entry point for our study was admission of the neonate, hence we did not test mothers antenatally and cannot determine the degree to which congenital infections were due to maternal re-activations or re-infections with multiple strains. HIV-exposed infants are known to suffer physical and mental developmental delay [28, 29], which in Zambia has been linked with CMV infection [18]. Importantly, the relationship between CMV and HIV appears to be bi-directional, with data from Thailand showing that congenital and post-natal CMV infections are strong independent correlates of mother-to-child transmission (MTCT) of HIV [30]. Congenital and/or early infant CMV infections may be an important contributing factor to MTCT of HIV, and developmental delay in HIV-infected and exposed African children, which must be investigated further through sufficiently powered longitudinal case-controlled studies.

Notes

Acknowledgments: N.M, M.B, L.C, and J.T conceived of and wrote the study protocols. M.B & N.M coordinated the study. L.C, N.M and M.Kap performed patient recruitment. J.T & M.Kab performed laboratory diagnostic tests. M.B performed the analysis and M.B, N.M, L.C and J.T wrote the first and final drafts. All authors contributed to writing of the manuscript. The authors would like to acknowledge logistical support of the Human Herpesvirus 6 Foundation, CA, U.S and HerpeZ (www.herpez.org), a local Zambian NGO that promotes herpesvirus research and collaborations in sub-Saharan Africa.

Financial support: Reagents for this study were supported by 'Kid's Here & There', Ohio, U.S. All personnel donated their time voluntarily.

Potential conflicts of interest: All authors declare no conflict of interests

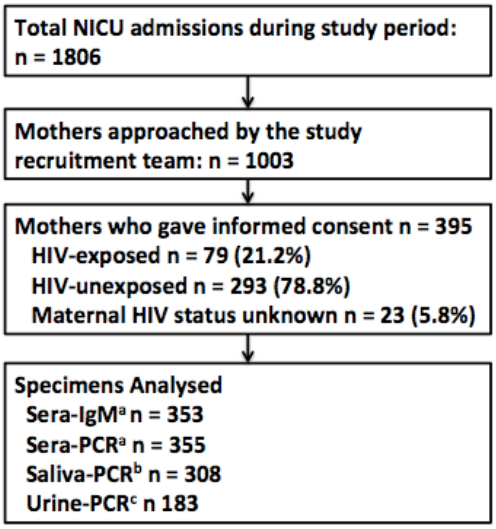
References

1. Mocarski ES, Jr; Shenk, T; Pass, R.F. Cytomegaloviruses. In: D.M Knipe PMHea. Fields Virology. 5 ed Vol. 2. Philidelphia: Lippincot, Williams and Wilkins, **2007**:2701-72.
2. Grosse SD, Ross DS, Dollard SC. Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment. J Clin Virol **2008**; 41(2): 57-62.
3. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988-2004. Clin Infect Dis **2010**; 50(11): 1439-47.
4. Tookey PA, Ades AE, Peckham CS. Cytomegalovirus prevalence in pregnant women: the influence of parity. Arch Dis Child **1992**; 67(7 Spec No): 779-83.
5. Schoub BD, Johnson S, McAnerney JM, et al. Is antenatal screening for rubella and cytomegalovirus justified? S Afr Med J **1993**; 83(2): 108-10.
6. Rodier MH, Berthonneau J, Bourgoin A, et al. Seroprevalences of Toxoplasma, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou, Republic of Benin. Acta Trop **1995**; 59(4): 271-7.
7. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med **1992**; 326(10): 663-7.
8. Stroffolini T, Ngatchu T, Chiaramonte M, et al. Prevalence of cytomegalovirus seropositivity in an urban childhood population in Cameroon. New Microbiol **1993**; 16(1): 83-5.
9. Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. J Clin Pathol **1991**; 44(5): 366-9.
10. Schopfer K, Lauber E, Krech U. Congenital cytomegalovirus infection in newborn infants of mothers infected before pregnancy. Arch Dis Child **1978**; 53(7): 536-9.
11. van der Sande MA, Kaye S, Miles DJ, et al. Risk factors for and clinical outcome of congenital cytomegalovirus infection in a peri-urban West-African birth cohort. PLoS One **2007**; 2(6): e492.
12. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev **2013**; 26(1): 86-102.
13. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, et al. Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. Clin Infect Dis **2009**; 49(4): 522-8.
14. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediatr Infect Dis J **2011**; 30(12): 1043-6.
15. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. N Engl J Med **1999**; 341(2): 77-84.

16. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. Pediatr Infect Dis J **1996**; 15(12): 1102-6.
17. Frederick T, Homans J, Spencer L, et al. The effect of prenatal highly active antiretroviral therapy on the transmission of congenital and perinatal/early postnatal cytomegalovirus among HIV-infected and HIV-exposed infants. Clin Infect Dis **2012**; 55(6): 877-84.
18. Gompels UA, Larke N, Sanz-Ramos M, et al. Human cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia. Clin Infect Dis **2012**; 54(3): 434-42.
19. Khansarinejad B, Soleimanjahi H, Mirab Samiee S, Hamidieh AA, Paryan M, Sanahmadi Y. Quantitation of human cytomegalovirus DNA in plasma using an affordable in-house qPCR assay. Journal of virological methods **2012**; 183(2): 170-5.
20. Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Rev Med Virol **2011**; 21(4): 240-55.
21. Albanna EA, El-Latif RS, Sharaf HA, Gohar MK, Ibrahim BM. Diagnosis of congenital cytomegalovirus infection in high risk neonates. Mediterranean journal of hematology and infectious diseases **2013**; 5(1): e2013049.
22. Tomasik T, Opozda A, Pietrzyk JJ. [Cytomegalovirus infection--diagnostic and therapeutic difficulties in neonatal intensive care unit]. Przeglad lekarski **2010**; 67(1): 18-24.
23. Clarke LM, Duerr A, Feldman J, Sierra MF, Daidone BJ, Landesman SH. Factors associated with cytomegalovirus infection among human immunodeficiency virus type 1-seronegative and -seropositive women from an urban minority community. J Infect Dis **1996**; 173(1): 77-82.
24. Schoenfisch AL, Dollard SC, Amin M, et al. Cytomegalovirus (CMV) shedding is highly correlated with markers of immunosuppression in CMV-seropositive women. Journal of medical microbiology **2011**; 60(Pt 6): 768-74.
25. Lurain NS, Robert ES, Xu J, et al. HIV type 1 and cytomegalovirus coinfection in the female genital tract. J Infect Dis **2004**; 190(3): 619-23.
26. Chandwani S, Kaul A, Bebenroth D, et al. Cytomegalovirus infection in human immunodeficiency virus type 1-infected children. Pediatr Infect Dis J **1996**; 15(4): 310-4.
27. Mostad SB, Kreiss JK, Ryncarz AJ, et al. Cervical shedding of cytomegalovirus in human immunodeficiency virus type 1-infected women. J Med Virol **1999**; 59(4): 469-73.
28. Makasa M, Kasonka L, Chisenga M, et al. Early growth of infants of HIV-infected and uninfected Zambian women. Trop Med Int Health **2007**; 12(5): 594-602.
29. Arpadi S, Fawzy A, Aldrovandi GM, et al. Growth faltering due to breastfeeding cessation in uninfected children born to HIV-infected mothers in Zambia. Am J Clin Nutr **2009**; 90(2): 344-53.
30. Khamduang W, Jourdain G, Sirirungsi W, et al. The interrelated transmission of HIV-1 and cytomegalovirus during gestation and delivery

in the offspring of HIV-infected mothers. J Acquir Immune Defic Syndr **2011**; 58(2): 188-92.

Figure 1: Flow diagram showing patient recruitment and sample collection



[a] Two sera specimens were minimal and used up in DNA-extraction for PCR analysis
[b] Saliva collection vials and transport medium were not available at the start of the study
[c] Urine collection proved challenging with patients either being discharged or dying before a sample could be collected

Figure 2: Stacked histogram showing prevalence of congenital CMV stratified by maternal HIV status and indicting symptomatic infections

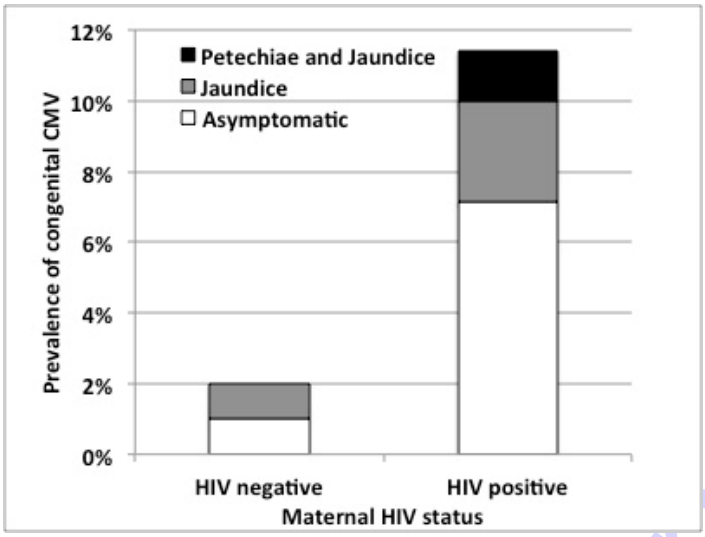


Table 1: Oligonucleotides and cycling conditions

Target	Oligo Name	Oligo sequence (5'-3')	Cycling Conditions
CMV UL83	UL83F UL83R UL83p	CAGTCCCGAGACMGTGAGAC TGAACATCCCCAGCATCAACG [HEX]TGCCACATCTGCTTGCCCGACGC[BHQ]	Hold: 95°C:10min Cycling: 95°C:10s , 58°C:20s, 72°C: 1s (45 cycles)
β-Actin	β-ActinF β-ActinR β-Actinp	CACACTGTGCCCATCTACGA CTCAGTGAGGATCTTCATGAGGTAGT [FAM]ATGCCCTCCCCCATGCCATCCTGCGT[TAMRA]	Hold: 94°C:3min Cycling: 94 °C:20s, 65°C:50s (45 cycles)

Table 2: Descriptive characteristics of the study group compared with the inpatient neonatal population

	Study (Nov 2012- Apr 2013)	**Population (June + July 2013)**	**Significance[a]**
	n (%)	n (%)	p
Female Gender	161 (41.7%)	280 (47.9%)	0.056
Pre-term Birth	135 (35.9%)	341 (58.5%)	<0.001
Birth Weight (mean, SD)	2.6, 0.9	2.2, 1.0	0.988[b]
Maternal HIV			
HIV positive	79 (21.2%)	112 (20.9%)	1
Mortality			
Died	65 (17.1%)	321 (55.0%)	< 0.001

[a]Paerson's chi squared in less otherwise indicated; [b]Mann Whitney U

Table 3: Detection and prevalence of congenital CMV infection

Patient	PCR Saliva[a]	PCR Urine[a]	PCR Sera[a]	IgM Sera	Positives
4	NA	41.4	Negative	Negative	+
5	NA	Negative	Negative	Positive	+
9	NA	26.5	35.8	Negative	++
20	Negative	NA	29.5	Negative	+
23	NA	40.9	Negative	Negative	+
45	Negative	Negative	38.2	Negative	+
100	Negative	NA	Negative	Positive	+
143	Negative	38.2	Negative	Negative	+
227	14.9	Negative	Negative	Negative	+
245	7.7	30.2	40.7	Negative	+++
265	Negative	35.4	36.2	Positive	+++
298	NA	42.2	Negative	Negative	+
340	Negative	NA	37.0	Negative	+
376	38.8	NA	Negative	Negative	+
384	Negative	35.01	Negative	Negative	+
Prevalence	0.97% (3/308)[b]	4.4% (8/183)[b,c,d]	1.7% (6/355)[c]	0.8% (3/353)[d]	NA
Median CT	14.9	36.8	36.6	NA	NA

[a] PCR positives indicated by CT value, [b] p = 0.017, [c] p = 0.062, [d] p = 0.01

Table 4: Binary logistic regression analysis

		Congenital CMV	Univariate Analysis		Multivariate Analysis[b]	
Neonatal Factors		**Proportion (%) [95%CI]**	**OR [95%CI]**	**Significance**	**OR [95%CI]**	**Significance**
Mean birth weight (SD)		2.5 (1.1)	0.864 [0.489-1.526]	0.615	1.341 [0.684-2.629]	0.393
Gender	Male	9/225 (4.0%) [2.1-7.4%]				
	Female	6/161 (3.7%) [1.7-7.9%]	0.929 [0.324-2.664]	0.891	0.908 [0.284-2.902]	0.870
Gestational Age	Term	9/241 (3.7%) [1.8-7.2%]				
	Pre-term	6/135 (4.4%) [1.8-9.8%]	1.199 [0.417-3.444]	0.736	0.715 [0.221-2.309]	0.575
Jaundice	No	8/317 (2.5%) [1.2-5.1%]				
	Yes	6/57 (10.5%) [4.4-22.2%]	4.544 [1.514-13.640]	0.007	5.701 [1.776-18.306][d]	0.003
Petechiae[c]	No	13/373 (3.5%) [2.0-6.0%]				
	Yes	1/1 (100%) [5.5-100%]	Not calculable		Not calculable	
Maternal Factors						
Age		27 (25-32)	1.033 [0.955-1.118]	0.420	0.992 [0.902-1.090]	0.867
HIV status	HIV uninfected	6/293 (2.1%) [0.8-4.6%]				
	HIV infected	9/79 (11.4%) [6.1-20.3%]	6.15 [2.119-17.849]	0.001	6.661 [2.126-20.876][d]	0.001
Education[a]	None	0/5 (0%) [0-53.7%]				
	Primary	5/106 (4.7%) [1.8-11.2%]				
	Secondary	4/175 (2.3%) [0.7-6.1%]	0.473 [0.124-1.8]	0.272	0.588 [0.148-2.343]	0.452
	Tertiary	3/49 (6.1%) [1.6-17.9%]	1.317 [0.302-5.748]	0.714	1.373 [0.225-8.389]	0.731
Marital Status	Single	11/316 (3.5%) [1.8-6.3%]				
	Married	1/45 (2.2%) [0.1-13.2%]	1.587 [0.2-12.593]	0.662	1.288 [0.153-10.843]	0.816
Mode of Delivery	SVD	11/277 (4.0%) [2.1-7.2%]				
	Caesarean	4/78 (5.1%) [1.7-13.3%]	1.307 [0.404-4.224]	0.655	2.132 [0.575-7.905]	0.258

[a] Maternal education was analysed with 'primary' as the reference category. The five mothers with no formal education were excluded from the analysis.
[b] Controlled for the effect of jaundice and maternal HIV status
[c] There was only one neonate with petechiae
[d] Disregarding the two IgM positive cases that were PCR negative, adjusted ORs were: 6.768 [1.994-22.967], p = 0.002, for maternal HIV infection and 5.281 [1.517-18.387], p = 0.009, for Jaundice
Abbreviations: OR = Odds Ratio, CI = Confidence Interval, SD = Standard Deviation