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Seroprevalence of CMV, HSV-2 and HBV among HIV-Infected Malawian Children: A Cross-sectional Survey

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ABSTRACT

Background: Little is known about viral co-infections in African human immunodeficiency virus (HIV)-infected children. We examined the prevalence of seromarkers for cytomegalovirus (CMV), herpes simplex virus type 2 (HSV-2) and hepatitis B virus (HBV) infections among HIV-infected, antiretroviral treatment (ART)-naïve children in Lilongwe, Malawi.
Methods: Ninety-one serum samples were tested for IgG and IgM antibodies to CMV, and IgG antibodies to HSV-2 and hepatitis B surface antigen (HBsAg). Baseline demographic, clinical and laboratory data were abstracted from electronic records.
Results: CMV IgG was the most common positive result in all age groups (in 73% of children <1 year, and 100% in all other groups). Three patients were CMV IgM positive (3.3%), suggesting acute infection. HSV-2 IgG was positive in four patients (4.4%), and HBsAg in two (2.2%).
Conclusions: CMV infection occurred early in life, and few children had specific signs of CMV infection at the time of ART initiation. Unrecognized HBV infection represents opportunities for testing and treatment of HIV/HBV co-infected children.

KEYWORDS: viral co-infections, HIV, children, CMV, HSV-2, HBV

BACKGROUND

Co-infections in people living with human immunodeficiency virus (HIV) activate the immune system and drive progression of their HIV infection [1]. In sub-Saharan Africa (SSA), HIV progression in children is faster than in comparable groups of children in Europe and the USA [2]. The causes for this phenomenon are not fully understood, but more frequent viral co-infections are likely to contribute [3]. However, few studies have reported seroprevalence of common viral co-infections among HIV-infected children in resource-limited settings. Specific

research regarding cytomegalovirus (CMV) [3–5], herpes simplex type 2 (HSV-2) [6] and hepatitis B virus (HBV) [3, 7–10] in SSA are scarce, despite the fact that the majority of children with HIV live there. In Malawi, data on seroprevalence of these viruses have been reported only in adults [11–13]. More regional data about HIV and other virus co-infection is urgently needed to better understand patients' clinical course and outcomes.

CMV acquisition is almost ubiquitous during infancy in SSA; data from The Gambia showed that approximately 85% of children were infected by 1 year of life [14]. In HIV-infected children, CMV appears to be even more common and 79% of children were infected by 6 weeks of age in Zimbabwe [4]. Furthermore, CMV replicates more rapidly in the presence of HIV, impacting neurodevelopment and hearing function [5]. HSV-2 seroprevalence has been reported to be three times higher among HIV-infected adults than in the general population [15]. HSV-2 infection often presents with oral and anogenital ulcers but can also cause serious systemic manifestations [16], particularly in neonates where HSV-2 infection has a poor prognosis [17].

Data on paediatric HBV/HIV co-infection from Malawi is lacking, but in adults it is common, with reported prevalence between 16% and 20% [11–13]. HBV/HIV co-infection has been associated with immune reconstitution syndrome following antiretroviral treatment (ART) initiation [18]. Hepatoxicity, progression of HBV-related liver disease and HBV drug resistance are other possible complications [18]. Furthermore, mother to child transmission of HIV (MTCT) can be affected not only by maternal but also by child co-infections including genital tract infection with HSV and systemic infections such as CMV and HBV, and adversely impact MTCT rates [19].

We aimed to investigate the seroprevalence of viral co-infections in a group of ART-naïve, HIV-infected Malawian children starting ART and relate our findings to specific age groups to add to the understanding of their epidemiology in this population.

METHODS

From the 410 HIV-infected children aged between 3 months and <15 years that started ART between May 2008 and December 2010 during the course of the Trioped study [20], 275 (67%) baseline serum samples were available. Epi Info 6 was used to determine study sample size using expected infection prevalence, and samples from 91 patients were randomly selected using Microsoft Excel Rand function. Testing was performed at the University of North Carolina (UNC) project laboratory. Demographic, clinical and other baseline data were obtained from electronic patient files. Levels of immunosuppression were defined according to the World Health Organization (WHO) definitions [21], anthropometric measures were calculated according to the WHO reference growth standards of 2007 using the WHO Anthro Plus Software version 1.0.4 for children 0–19 years old [22]. Severe wasting was defined as a body mass index (BMI) for Age Z-score < -3 standard deviation (SD), and stunted was defined as a Height for Age Z-score < -2 SD, according to the classification standards of the WHO guidelines [23]. Age- and sex-specific reference ranges were used for aspartate transaminase (AST) and alanine transaminase (ALT) (Appendix 1).

All samples were screened for CMV IgG and IgM. CMV IgG antibodies were tested with the Bio-Rad Platelia CMV IgG enzyme-linked immunoabsorbent assay (ELISA) (Bio-Rad, Marnes-la-Coquette, France). We compared the optical density (OD) of the sample with the concentration in Arbitrary Units per millilitre (AU/ml) of the calibrators of the standard curve [OD = function (AU/ml)] to interpret the results. For CMV IgM antibodies, we used the Bio-Rad Platelia CMV IgM ELISA. Sample Ratios [sample OD/cut-off value (CO)] of $\geq$1.10 were considered positive for IgM antibodies to CMV. All samples were tested for HSV-2 antibodies against the HSV-2 G2 protein using the Bio-Rad Platelia HSV-2 IgG ELISA. Reactive samples with a sample ratio (sample OD/CO) $\geq$1.10 were considered anti-HSV-2 positive. Hepatitis B surface antigen (HBsAg) was examined with the Genetic Systems™ HBsAg enzyme immunoassay 3.0 (Bio-Rad, Redmond, USA) and samples with an OD above the specific CO for each test run were considered to be HBsAg positive. Table 1 shows the interpretation of positive test results for each viral marker.

The study was approved by National Health Sciences Research Committee, Lilongwe, Malawi, the Baylor College of Medicine institutional review board, Houston, Texas, USA, and the Liverpool School of Tropical Medicine Research Ethics Committee, United Kingdom.

RESULTS

Of the 91 HIV-infected children, 46 (50.5%) were female. The median age was 34 months, with a range of 3–165 months [interquartile range (IQR): 63]. The age distribution of the study population was 15 children (16.4%) <12 months, 41 (45.1%) 1–4 years, 29 (31.9%) 5–9 years and 6 (6.6%) 10–14 years. Two-thirds of the children had advanced WHO clinical stages 3 or 4 (Table 2). The mean absolute CD4 of the total sample was 682 cells/mm^3 (SD: 707.1) with a mean CD4 percent of 16% (SD: 9.2). Almost three in four children had advanced or severe immunosuppression (Table 2). The median viral load was 191,363 copies/mm^3 (IQR: 27,678). With regard to transaminases, the median AST was 41 U/l (IQR: 23) and the median ALT was 24.8 U/l (IQR: 17). Of the children included in the study, 21 (23.1%) and 25 (27.5%) children presented with an elevated level of AST and ALT, respectively (Table 2). In relation to nutritional status, 71.4% were stunted (65 of 91) and 9.9% (9) were severely wasted (Table 2).

CMV IgG was positive in 87 children (95.6%, 95% CI: 88.6–98.3), CMV IgM in 3 (3.3%, 95% CI: 1.1–9.9), HSV-2 IgG in 4 (4.4%, 95% CI: 1.6–11.3)

Table 1. Interpretation of positive test results

CMV IgG	Passive maternal transfer (if <12 months) or lifelong persistent infection (if ≥12 months)
CMV IgM	Primary infection or reactivation
HSV-2	Passive maternal transfer (if <12 months) or lifelong persistent infection (if ≥12 months)
HBsAg	Acute or chronic HBV infection

Table 2. Clinical and laboratory variables by age group

Variable	<1 year	1 to <5 years	5 to <10 years	10 to <15 years	Total/overall
Viral Load median copies/mm^3 (IQR)	332 917 (517 591)	254 769 (424 261)	102 480 (205 722)	76 873 (57 372)	191 363 (27 678)
Immunosuppression, *n* (%)					
No	0	5 (12.2)	7 (24.1)	1 (16.7)	13 (14.3)
Mild	1 (6.7)	2 (4.9)	5 (17.3)	1 (16.7)	9 (9.9)
Advanced	1 (6.7)	2 (4.9)	7 (24.1)	1 (16.7)	11 (12.1)
Severe	11 (73.3)	30 (73.1)	9 (31)	3 (50)	53 (58.2)
No data	2 (13.3)	2 (4.9)	1 (3.5)	0	5 (5.5)
WHO clinical stage, *n* (%)					
1	6 (40)	4 (9.7)	2 (6.9)	0	12 (13.2)
2	2 (13.3)	8 (19.5)	9 (31)	0	19 (20.9)
3	5 (33.4)	27 (65.9)	15 (51.7)	0	50 (54.9)
4	2 (13.3)	2 (4.9)	3 (10.4)	6 (100)	10 (11.0)
Elevated ALT[a] *n* (%)	0	12 (29.3)	10 (34.5)	3 (50)	25 (27.5)
Elevated AST[a] *n* (%)	3 (20)	9 (22)	8 (27.6)	1 (16.7)	21 (23.1)
Nutritional status[b]					
Stunted *n* (%)	12 (80)	28 (68.3)	19 (65.5)	6 (100)	65 (71.4)
Severely wasted *n* (%)	3 (20)	4 (9.8)	0	2 (33.3)	9 (9.9)

Notes. [a]Age- and sex-specific AST and ALT reference ranges from the UNC laboratory were used (Appendix 1).
[b]Severe wasting was defined as BMI for Age Z-score (BAZ) < −3 SD and stunted was defined as a Height for Age Z-score < −2 SD.

Table 3. Seropositivity for CMV, HSV-2 and HBsAg in HIV-infected children by age group

Age group Viral marker	<1 year *n* (%)	1 to <5 years *n* (%)	5 to <10 years *n* (%)	10 to <15 years *n* (%)	Total *n* (%, 95% CI)
CMV IgG *n* (%)	11 (73.3)	41 (100)	29 (100)	6 (100)	87 (95.6, 88.6–98.3)
CMV IgM *n* (%)	2 (13.3)	1 (2.4)	0	0	3 (3.3, 1.1–9.9)
HSV-2 IgG *n* (%)	0	2 (4.9)	2 (6.9)	0	4 (4.4, 1.6–11.3)
HBsAg *n* (%)	0	0	1 (3.4)	1 (16.7)	2 (2.2, 0.5–8.5)

and HBsAg in 2 (2.2%, 95% CI: 0.5–8.5) (Table 3). All children positive for CMV IgM, HSV-2 IgG and HBsAg also had CMV IgG antibodies. No child had more than two positive seromarkers. Only four children (4.4%) were negative for all tests. Eleven (73.3%) infants <1 year of age and all other children were positive for CMV IgG (Table 3).

Only one child positive for CMV IgM had the triad of fever, hepatomegaly and generalized lymphadenopathy (GL) at the time of ART initiation. This patient also had a history of severe recurrent pneumonia leading to a WHO stage 3 classification. At baseline, none of the children positive for CMV IgM or CMV IgG antibodies had the diagnoses of colitis, retinitis or encephalopathy, and no child infected with HSV-2 had documented skin lesions, hepatomegaly or GL. Among children with HBV infection, one child presented with GL; none had jaundice or hepatomegaly documented at ART initiation.

Liver transaminases (AST and ALT) were all within age- and sex-specific normal ranges for the children who were CMV IgM positive. One of the patients who was HBsAg positive did have an ALT that was 1.1 times the upper limit of normal.

DISCUSSION

Our study adds important local data about the seroprevalence of clinically relevant viral co-infections in HIV-infected children in a geographical area where evidence is scarce and variable. We found that all 76 children >1 year of age had serological evidence of CMV infection, and 11 of the 15 infants <12 months were either CMV exposed or infected. Three children had serological markers indicative of either acute or reactivated CMV infection, but only one had clinical features suggestive of CMV infection at ART initiation (in the setting of limited capacity to diagnose infections such as CMV retinitis or colitis in Malawi). Fewer children had laboratory evidence of HSV-2 or HBV infection (4.4 and 2.2%, respectively).

CMV infection has been reported to be an early-life event in Africa, promoted by HIV [4, 5] and our findings support this, with all children infected by 1 year of age. The HSV-2 prevalence in our sample is much lower than the 29.6% reported in a study from Tanzania [6] but similar to findings in HIV-uninfected children in Germany where the prevalence was <5% [24]. HBsAg positivity in HIV-infected children in Africa varies widely, with studies reporting prevalence from 1.2% to 7.8% [3, 7–10]. The seroprevalence of HBsAg in our study is similar to studies in HIV-positive children in Ethiopia (2%), the Democratic Republic of Congo (1.6%) or Tanzania (1.2%) [7–9], but lower than in studies from Kenya and Nigeria reporting 4% and 7.8%, respectively [3, 10]. Different baseline conditions, study design and selection bias are potential causes of the differences in the seroprevalence of these viral co-infections.

Routine HBV screening for all HIV-infected patients is recommended by the WHO [25], but has not been implemented in Malawi or many SSA countries [7, 26]. It is particularly important in paediatrics where an estimated 25% of children and infants co-infected with HIV and HBV will develop cirrhosis and carcinoma [27]. Evidence of previously unrecognized acute or chronic HBV infection in HIV-infected children (2.2% in this study) highlights a gap in the diagnosis of this co-infection in children. As of December 2014, the Malawian Ministry of Health reported 46,410 children (<15 years) were alive and on ART [28]. Using the 2.2% (95% CI: 0.5–8.5) HBsAg positivity from this study, approximately 1016 (between 232 and 3945) Malawian children on ART may have

undiagnosed HBV co-infection. This is a substantial number, and improvement in universal HBV screening in HIV-infected patients is needed. In addition, implementation of universal maternal HBV screening as part of routine antenatal care would allow for perinatal vaccination for prevention of vertical HBV transmission.

With respect to HBV treatment, the standard national paediatric first-line ART regimens over the period from 2004 to present has been zidovudine or stavudine with lamivudine plus nevirapine or efavirenz, with WHO-recommended dosing [29, 30]. Of these medications, only 3TC is effective against HBV, and 3TC monotherapy for HBV is associated with rapidly developing resistance [18]. Tenofovir is another antiretroviral with activity against HBV and became available for general use in Malawi for all HIV-infected patients in 2013 [31]. Current national guidelines allow for its use in children >35 kg and >3 years of age [26], but the drug has been also approved by the Food and Drug Administration for children over 2 years and greater than 10 kg [32]. While more widespread use of tenofovir for treatment of paediatric HIV may be limited owing to concerns about renal and bone side effects, guideline revisions should be considered to allow for situational use of this medication in younger, smaller children with known HIV-HBV co-infection, who could be identified in the future through expanded standard screening programs.

LIMITATIONS

This study has a number of limitations. First, the small sample size allows only a descriptive analysis. The study was not powered to find associations between seroprevalence and other parameters; and the cross-sectional design prevented us from analysing risk factors. Secondly, no information about exposure and likely transmission routes of viral co-infections was available. Thirdly, serologic laboratory tests have limitations in that passively transferred maternal CMV or HSV-2 IgG antibodies can persist in the child's circulation up to the age of approximately 12 months and do not necessarily represent infection in the child. Interpretation of negative antibody test results is difficult when tests are performed before sufficient antibody is produced or when antibody production is impaired. Furthermore, HSV-2 IgG antibodies do not distinguish between active and latent infection, and there is evidence that the sensitivity and specificity of HSV-2 tests used in SSA vary, and are generally lower than in studies from the USA and Europe [33]. Finally, HBsAg measurement underestimates the HBV prevalence as patients with negative HBsAg can be HBV DNA positive [34].

CONCLUSIONS

CMV infection early in life was common in this cohort of HIV-infected children initiating ART, but of unclear clinical significance. Acute or chronic HBV infection was much less prevalent, but opportunities for screening and treatment are currently being missed. Policy and practice changes to improve outcomes in HBV/HIV co-infected children are needed.

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APPENDIX

Transaminases levels of reference

Table A1. Normal levels of reference of ALT by age and sex at UNC-project laboratory (in U/l)

Alanine aminotransferase	U/l	5–20	10 years to <18 years F
		5–30	10 years to <18 years M
		5–25	4 years to <10 years F
		5–25	7 years to <10 years M
		5–20	4 years to <7 years M
		5–30	1 year to <4 years
		13–45	1 day to <1 years

Table A2. Normal levels of reference of AST by age and sex at UNC-project laboratory (in U/l)

Aspartate aminotransferase	U/l	15–45	12 years to <18 years
		10–60	10 years to <12 years
		15–40	7 years to <10 years
		15–50	4 years to <7 years
		20–60	1 year to <4 years
		15–60	>30 days to <1 years
		25–75	0 day to 30 days