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Original Article **Open Access**

Cytomegalovirus co-infection with human immunodeficiency virus among children in State Hospital, Ibadan, Nigeria

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Abstract:

Background: Cytomegalovirus (CMV) co-infection with human immunodeficiency virus (HIV) poses significant global challenges with possible complications of disease progression in children and adolescents, especially in resource-limited settings. This study investigates the prevalence of CMV among children living with HIV as well as their viral load dynamics and immunological status correlates.
Methodology: A total of 169 HIV infected participants, including 37 children (2 months to 10 years) and 132 adolescents (11–19 years) on antiretroviral therapy (ARTs), were enrolled from the State Hospital HIV Clinic, Ibadan, Nigeria. The CMV-DNA cycle threshold (Ct) and HIV viral loads were determined using real-time polymerase chain reaction (rt PCR) assay while $CD4^+$ cell counts were assessed with CyFlow method. Socio-demographic and clinical data of participants were analyzed using SPSS (version 25.0) and statistical comparisons and correlations between variables were evaluated at significant level of $p<0.05$.
Results: The mean age of all HIV-infected participants was 11.89±3.16 years, and were predominantly males (65.1%, n=110). The overall prevalence of CMV was 13.0%, with higher rates among males (20.9%) compared to females (5.4%) (OR=3.897, 95% CI=1.103-13.776, $p=0.03$). The prevalence of low and high CMV DNA Ct were 8.9% and 4.1% respectively. Most of the participants had low HIV loads (<19 copies/μL) (n=102, 60.4%) and high $CD4^+$ counts (>500 cells/μL) (n=134, 79.3%). However, a subset of the participants (n=12, 7.1%) had high CMV DNA levels despite their low HIV loads. A negative correlation between CMV DNA Ct values and age ($r<0$) suggested increased CMV loads in older participants.
Conclusion: Cytomegalovirus co-infecting with HIV is present at a prevalence of 13.0% in this study, and significantly higher in males and older age groups, depicting a significant concern among male children and adolescents living with HIV. These findings highlight the need for routine CMV screening among children and adolescents living with HIV, age- and gender-sensitive interventions, as well as enhanced ART adherence, to mitigate the risks associated with co-infection in this vulnerable population.

Keywords: CMV; HIV; co-infection; real time PCR; cycle threshold; $CD4^+$ T cell

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Co-infection par le cytomégalovirus et le virus de l'immunodéficience humaine (VIH) chez des enfants à l'hôpital public d'Ibadan, au Nigéria

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Résumé:

Contexte: La co-infection par le cytomégalovirus (CMV) et le virus de l'immunodéficience humaine (VIH) pose des défis mondiaux importants, avec un risque potentiel complications de la progression de la maladie chez les enfants et les adolescents, en particulier dans les milieux à ressources limitées. Cette étude examine la prévalence du CMV chez les enfants vivant avec le VIH ainsi que la dynamique de leur charge virale et les corrélats de leur statut immunologique.
Méthodologie: Un total de 169 participants infectés par le VIH, dont 37 enfants (2 mois à 10 ans) et 132 adolescents (11-19 ans) sous traitement antirétroviral (TAR), ont été recrutés à la clinique VIH de l'hôpital d'État d'Ibadan, au Nigéria. Le seuil du cycle CMV-ADN (Ct) et les charges virales VIH ont été déterminés à l'aide d'un test de réaction en chaîne par polymérase en temps réel (rt PCR) tandis que le nombre de cellules $CD4^+$ a été évalué avec la méthode CyFlow. Les données sociodémographiques et cliniques des participants ont été analysées à l'aide de SPSS (version 25.0) et les comparaisons statistiques et les corrélations entre les variables ont été évaluées à un niveau significatif de $p<0,05$.
Résultats: L'âge moyen de tous les participants infectés par le VIH était de 11,89±3,16 ans et il s'agissait principalement d'hommes (65,1%, n=110). La prévalence globale du CMV était de 13,0%, avec des taux plus élevés chez les hommes (20,9 %) que chez les femmes (5,4%) (OR=3,897, IC à 95%=1,103-13,776, $p=0,03$). La prévalence d'un Ct d'ADN CMV faible et élevé était respectivement de 8,9% et 4,1%. La plupart des participants avaient une faible charge de VIH (< 19 copies/µL) (n=102, 60,4%) et un taux élevé de $CD4^+$ (>500 cellules/µL) (n=134, 79,3%). Cependant, un sous-ensemble de participants (n=12, 7,1%) avait des taux élevés d'ADN CMV malgré leur faible charge de VIH. Une corrélation négative entre les valeurs de Ct de l'ADN du CMV et l'âge ($r<0$) suggère une augmentation des charges de CMV chez les participants plus âgés.
Conclusion: La prévalence de la co-infection par le cytomégalovirus et le VIH est de 13,0% dans cette étude, et significativement plus élevée chez les hommes et les groupes d'âge plus avancés, ce qui témoigne d'une préoccupation importante chez les enfants et les adolescents de sexe masculin vivant avec le VIH. Ces résultats soulignent la nécessité d'un dépistage systématique du CMV chez les enfants et les adolescents vivant avec le VIH, d'interventions tenant compte de l'âge et du sexe, ainsi que d'une meilleure observance du traitement antirétroviral, afin de réduire les risques associés à la co-infection dans cette population vulnérable.

Mots-clés: CMV; VIH; co-infection; PCR en temps réel; seuil de cycle; lymphocytes T $CD4^+$

Introduction:

Viral co-infections with human immunodeficiency virus (HIV) are common findings in infected individuals especially the viruses with similar route of infection, and they are implicated as a contributory factor to the progression of disease development (1). Some of the co-infections can be silent without notable impact on HIV disease progression while HIV co-infection with certain viral infections can affect the natural history of HIV infection leading to severe complications. Among the infectious agents that can disrupt the natural history and course of HIV infection towards severe diseased state when co-infecting the same host are hepatitis C virus (HCV), hepatitis B virus (HBV), human T-cell lymphotrophic virus-1 (HTLV-1), human herpes virus-8 (HHV-8), human papilloma virus (HPV), cytomegalovirus (CMV) and others (1,2).

HIV remains a significant public health concern, particularly in sub-Saharan Africa, accounting for two-thirds of the overall world HIV infections, where the burden of the disease is disproportionately high among children (3). In Nigeria, pediatric HIV presents unique challenges, especially when complicated by co-infections (4). One such co-infection is cytomegalovirus (CMV), a common herpes virus (human herpes virus-5) that poses serious risks to immunocompromised individuals, including children living with HIV (5).

Cytomegalovirus is a highly prevalent viral pathogen belonging to the beta herpesvirinae subfamily, known for its ability to establish lifelong latency in the host. CMV infection is widespread globally, but its impact is most profound in regions with lower socioeconomic status (6). While CMV is often asymptomatic in healthy individuals, it poses significant risks to immunocompromised poplations, notably in those living with HIV, and it can lead to severe complications such as retinitis, esophagitis, encephalitis, hepatitis and pneumonia amongst others (7). CMV is primarily transmitted through bodily fluids such as saliva, urine, breast milk, and blood, with infants often acquiring the virus through breastfeeding, delivery via the birth canal, or in-utero. The virus can also spread among young children through contact with contaminated saliva, in settings where shared toys and objects are common (8).

The use of combination antiretroviral therapy (cART) has drastically reduced the incidence of CMV-related complications in HIV positive individuals, especially those with severe immunosuppression. Before the widespread availability of ART, approximately 40% of severely immunocompromised HIV-positive individuals experienced symptoms of CMV during their lifetime (9). Nevertheless, the effectiveness of ART has not eliminated the need for awareness and early screening for CMV, as this continues to pose a serious risk, more so in regions where access to healthcare remains limited.

Despite the known detrimental effects of CMV co-infection in HIV-positive individuals, there is a paucity of data on its prevalence, impact, and the demographic factors

influencing its occurrence in pediatric populations and effective management strategies in Sub-Saharan Africa (10).

This study is designed to determine the prevalence of CMV among children living with HIV in Ibadan, Nigeria, and to evaluate the impact of antiretroviral therapy (ART) on their immune function through viral load and $CD4^+$ T cells treatment indices. The findings from this study aimed to contribute to the understanding of how CMV affects HIV-positive children, clinical practice and highlight the importance of regular screening of CMV among vulnerable populations.

Materials and method:

Study setting, design and participants:

This was a hospital based cross-sectional study, which recruited a total of 169 participants comprising 37 children (2 months to 10 years) and 132 adolescents (11-19 years) from September 2023 to August 2024. The participants were made up of 110 males and 59 females, who were confirmed to be HIV positive by serology and PCR, already on antiretroviral therapy (ART) and accessing care at the out-patient HIV clinic of the State Hospital, Ibadan, Nigeria.

Laboratory analyses were performed at the Biorepository and Clinical Virology Laboratory (BCVL) and the Haematology laboratory of the Department of Biomedical Laboratory Science (BMLS), College of Medicine, University of Ibadan.

Ethical consideration:

Ethical approval was obtained from Oyo State Ethics Research Committee, Ministry of Health, Ibadan, Nigeria (NHREC/OYO SHRIEC/10/11/22) and informed consents were obtained from the parents/caregivers of the children while those above 12 years were given child assents form before being enrolled into the study.

Inclusion and exclusion criteria:

The inclusion criteria were confirmed positive HIV status, ART initiation and accessing care at the State Hospital, while the exclusion criteria were non-consenting individuals, children without HIV infection and children experiencing a current ongoing illness.

Data and sample collection, handling and processing:

The socio-demographic data and clinical information of the participants were collected using interviewer administered questionnaires and abstraction of data from patients' case notes. Venous blood samples (5ml) were aseptically collected by a professional Phlebotomist into two sterile EDTA sample bottles from the participants and transported to the Clinical Virology Laboratory where aliquot of blood samples from the first bottle was used for viral load analysis and $CD4^+$ T cell count. Blood samples in the second bottle was centrifuged, and the plasma separated and stored at -20°C for CMV DNA molecular analysis.

$CD4^+$ T cell count:

The $CD4^+$ T cell count was measured using automated Sysmex Partec Cyflow device with $CD4^+$ Monoclonal easy count kit (Sysmex-Partec Germany).

Molecular analysis of samples for CMV and HIV detection:

Nucleic acid extraction:

DNA and RNA were extracted from 200 µL of each plasma sample obtained from the participants whole blood using the Qiagen RNA/DNA Extraction Kit, which has a specific polymer group that absorbs nucleic acid from the samples.

Quantitative detection of CMV DNA by real time fluorescence PCR:

The presence of CMV DNA and HIV 1 RNA were detected in the extract using commercially available kit (Guangdong Huayin Medicine Science Co., Ltd.). Amplification of the extracted nucleic acid was performed on a multiple fluorescent real-time PCR technology to quantitatively detect HIV-1 and HCMV DNA levels in samples using specific CMV primers and probes, with an internal control to monitor PCR inhibitor levels and prevent false negative PCR.

The PCR reaction tube, CMV PCR Master Mix, which contains the CMV primers and probes (forward primer: 5'-GAGCCTTTGGAG GA-3' and reverse primer: 5'-GGCTGAGTTCT TGG-3'), along with reaction buffers, Mg^{2+}, dNTPs, Taq DNA Polymerase, was mixed with the extracted DNA samples, CMV positive control (recombinant plasmid containing target gene), negative control (physiological saline) and redissolved diluent (purified water). The reaction tube was placed in the automatic fluorescent PCR instrument and PCR performed in accordance with the operating instructions of the instrument. After the reaction was completed, the results were automatically saved. The start, end and threshold values of the baseline were adjusted according to the analysed image.

The cycle threshold (Ct) value was used as proxy for HCMV detection (qualitative rt PCR), with Ct>0.00 as positive (≥200 IU/ml), Ct=0.00 as negative (<200 IU/ml), Ct< 30 ($1.00x10^3$-$1.00x10^8$ IU/ml) as high positive and Ct>30 (≥200 IU/ml-$1.00x10^3$IU/ml) as low positive.

Data analysis:

Data were analyzed using Statistical Package for Social Sciences (SPSS version 25.0). Descriptive statistic was used to summarize the qualitative (categorical) data. The quantitative data were presented as mean± standard deviation ($\bar{x}$±SD), median (interquartile range) and rates.

The prevalence of CMV co-infection was determined, and the mean HIV loads was compared between CMV-positive and CMV-negative participants. Correlation analysis was performed to explore the relationship between ART duration, HIV load, and CMV positivity. A *p*-value of <0.05 was considered significant.

Results:

Socio-demographic characteristics of the participants:

The participants had a mean age of 11.89±3.16 years with age range of 2 months-19 years. The age group 11-15 years has the highest frequency, followed closely by age group 6-10 years while those in age group 6-19 years were the lowest in frequency. The male participants were predominant and the participants had a mean weight of 32.48kg (Table 1).

Table 1: Demographic data and anthropometric measurements of the study participants

Variable	Frequency (%)
Age group	
2 months – 5 years	12 (7.1)
6-10 years	25 (14.8)
11-15 years	127 (75.1)
16-19 years	5 (2.0)
Mean age ± SD (years)	11.89±3.16
Mean weight ± SD (kg)	32.48±10.59
Gender	
Male	110 (65.1)
Female	59 (34.9)

HIV load, CD4$^+$ cell count and HCMV cycle threshold values of the participants:

Table 2 displayed the mean HIV load and CD4$^+$ T-cells count as well as HCMV cycle threshold (Ct) of all the participants. The mean Ct of HCMV-DNA for all the positive participants is 2.52±0.65, while the mean Ct of HCMV for participants with Ct<30 (high) is 10.97±8.85 (range 1.26-29.49) and for participants with Ct>30 (low) is 37.47±11.08 (range 31.96-39.59) (p<0.05). The mean HIV-1 RNA viral load for all the study participants is 21250.80±11271.32 while the mean CD4$^+$ T cells is 893.84±520.37.

Table 2: Mean of CD4$^+$ T- cells count, HIV-1 RNA load and HCMV DNA Cycle threshold values among the study participants

Variable	Mean± SD
CD4$^+$ T cell count (cells/µL)	893.84±520.37
HIV load (copies/mL)	21250.80±11271.32
HCMV DNA Ct value (Total)	2.52±0.65
HCMV DNA Ct value <30 (1.26-29.49)	10.97±8.85*
HCMV DNA Ct value >30 (31.96-39.59)	37.47±11.08*

CD4- Cluster of Differentiation 4; HCMV DNA Ct- Cytomegalovirus deoxyribonucleic acid Cycle threshold. *p-value significant at ≤0.05

Analysis of prevalence of HCMV with characteristics of the study participants:

Table 3 shows HCMV prevalence of 13.0% (22/169) in the study participants with high and low HCMV prevalence of 8.9% and 4.1%, respectively. Most of the study participants had low HIV-1 RNA viral load (<19 copies/mL) (60.4%, 102/169) and high CD4$^+$ T cell count (>500 cells/µL) (79.3%, 134/169).

Table 3: Distribution and prevalence of HCMV, HIV-1 RNA viral load and CD4$^+$ T cell count values in the participants

Parameters	Frequency (%)
HCMV DNA	
Positive (Ct<30 - high)	15 (8.9)
Positive (Ct >30 - low)	7 (4.1)
Positive Ct > 0.00 -Total)	22 (13.0)
Negative Ct = 0.00	147 (87.0)
HIV-1 RNA Viral Load (copies/mL)	
≤19	102 (60.4)
20-200	19 (11.2)
>200	48 (28.4)
CD4$^+$ T cell count (cells/µL)	
<200	10 (5.9)
200-500	25 (14.8)
>500	134 (79.3)

CD4-Cluster of Differentiation 4; HCMV DNA- Cytomegalovirus deoxyribonucleic acid; Ct - Cycle threshold

The prevalence of HCMV in the HIV infected children by gender is 5.4% (3/56) and 20.9% (19/91) in the female and male participants respectively (OR=3.897, 95% CI =1.103-13.776, p=0.03) (Fig 2). Fig 2a compared the distribution of participants levels of HIV-1 RNA viral load with HCMV DNA Ct values, which shows that those with zero HCMV DNA Ct values were the majority with low (<19 copies/mL), intermediate (20-200 copies/mL) and high (>200 copies/mL) HIV-1 RNA viral load (p<0.05). However, there were notable participants (n=12, 7.1%) with both low HCMV DNA Ct value (high HCMV level) and low HIV load.

Fig 2b compared the distribution of the HIV-1 RNA and HCMV DNA Ct values where majority of those with zero HCMV DNA

had high CD4⁺ T cell count (>500 cells/µL), others had intermediate (200 - 500 cells/µL) and low CD4⁺ T cell count (< 200 cells/µL), while all participants with positive HCMV DNA at low or high Ct values had a high CD4⁺ T cell count.

Fig 3 shows the correlation of HCMV DNA Ct values with the age of the participants, with a moderate negative correlation ($r<0$) which inferred that as the age increases, the HCMV Ct value decreases thereby implying increased HCMV load in older participants.

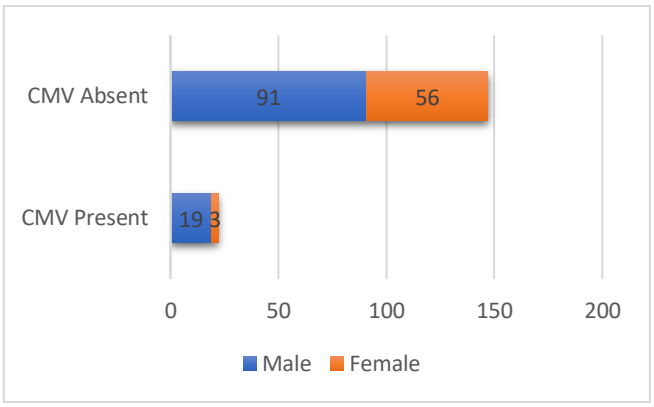
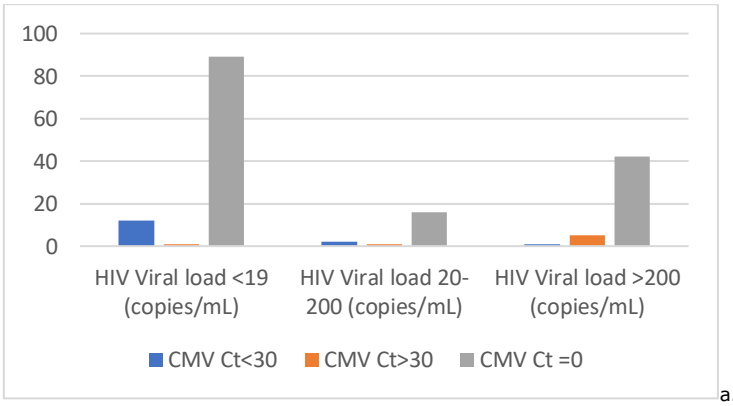
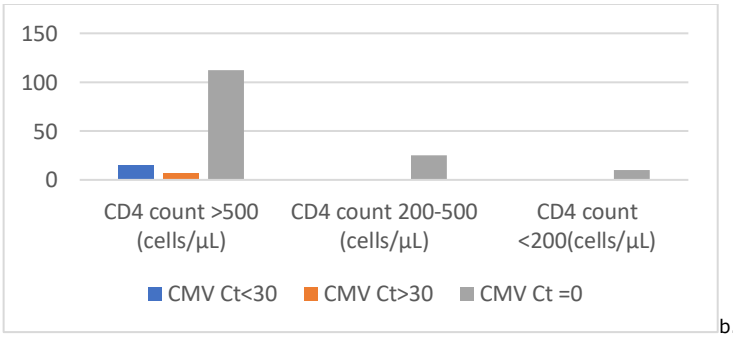


Fig 1: Prevalence of CMV by gender among the study participants



a.



b.

Fig 2: Comparison of distribution of HCMV DNA Ct value with **a:** HIV-1 RNA viral load **b:** CD4 T-cells count.

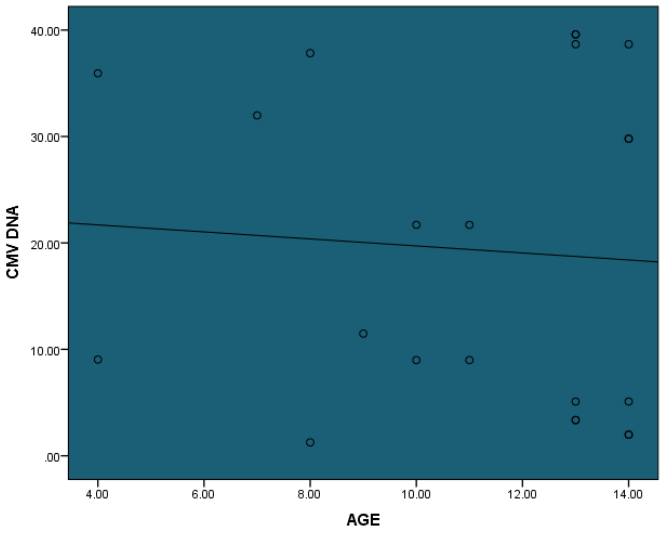


Fig 3: Correlation of HCMV DNA Ct values with the age of the study participants

Discussion:

The co-infection of CMV can be a major complication in children with HIV and could be associated with increased risk of AIDS-defining criteria leading to higher mortality and morbidity. In this study, the socio-demographic results of the mean age and broad age range of the participants displayed the predominance of older children and adolescents in HIV clinics thereby reflecting the success of earlier pediatric HIV interventions that enable longer survival into adolescence. This aligned with previous studies on paediatric and adolescent HIV population described by Mwaanza et al., (11) and by WHO (12), where children aged 10-15 years have been estimated to be the highest population with HIV by the end of the year 2023. The lower representation in the age-group 16–19 years may be indicative of attrition due to delayed diagnosis, ineffective treatment or treatment interruptions which are concerns previously noted in studies examining survival trends among HIV-positive adolescents (13,14).

Also, this study revealed that the male outnumbered female participants, which may be as a result of regional differences in health-seeking behaviors or sampling variations. This is in contrast with some other reported observation where females showed dominance among adult and pediatric HIV cohorts (15). In addition, the mean weight of 32.48 kg reflected the nutritional and health status of the cohort depicting an average low weight of the participants which could be attributable to growth delays and nutritional deficiencies in HIV-positive children despite being on ART. Similar findings were reported in some Nigeria-based studies where HIV-positive children and adolescents in the country demonstrated weight ranges indicative of chronic disease impact, despite ART (16,17).

In this study, the prevalence of HCMV is 13.0% in the children and adolescents' participants who were living with HIV. This aligned with findings from similar studies conducted among HIV-positive pediatric populations ranging from 10% to 33%, reflecting the endemic nature of HCMV in regions with high HIV burdens (2,8,18). The mean CMV Ct value of 2.52±0.65 further highlighted the sensitive detection method resulting in display of active replication of CMV represented by the PCR Ct values within the population, which is suggestive of the need for routine CMV screening among HIV-positive individuals especially the children and adolescents to prevent complication of disease progression.

Furthermore, the distinction between the high (8.9%) and low (4.1%) HCMV DNA PCR Ct level is suggestive of the heterogeneity in viral replication among those with detectable HCMV DNA. The participants with lower HCMV Ct levels indicated a corresponding high CMV DNA viraemia in the system, who may therefore be at increased risk for CMV-associated complications, such as immune activation and end-organ disease, particularly if their immune system is not fully restored by ART. However, those with high PCR Ct level, indicating HCMV DNA load were low, are the majority in the participants, hence it poses a reasonable explanation for most of the participants having a good status of HIV-1 RNA viral load and $CD4^+$ T cell count.

The high number of participants with low HIV viral load and high $CD4^+$ T cell levels explained success of the various ARTs in achieving viral suppression and immune recons-

titution among the participants in the Ibadan, Nigeria. The group represented individuals whose HIV infection were being effectively managed, thereby reducing their susceptibility to opportunistic infections such as CMV. Similar pattern was reported in the study by Albasanz-Puig et al., (19) where the prevalence of CMV-specific immune response was high and endured over time despite the presence of CMV viraemia. However, participants with contrasting level of higher HIV loads and lower $CD4^+$ T cell count reflected a population at greater risk of co-infection complications which cut across individuals with ART non-adherence, treatment failure, or advanced diseases, therefore stressing the need for targeted interventions to improve outcomes.

In furtherance to the prevalence of CMV in the HIV infected pediatric participants, there was an observed significant disparity in CMV prevalence between genders, with 5.4% in females and 20.9% in males (p=0.03). This highlighted an important variation which could be majorly traced to the higher male frequency gender distribution in the entire population, as well as reports of higher male susceptibility to CMV reactivation or acquisition due to biological, behavioral, or environmental factors. These were considered based on similar reports from other studies among paediatric populations with and without HIV infection (8,20-23).

Additionally, most participants without detectable CMV DNA Ct had low (<19 copies/mL), intermediate (20–200 copies/mL), and high (>200 copies/mL) HIV loads, an observation that stresses the effectiveness of ART in suppressing HIV replication and reducing the risk of CMV reactivation (19,24-26). However, the presence of a notable subset (n=12 participants) with both low CMV DNA Ct values (indicating high CMV levels) and low HIV loads suggests a unique interaction between these infections which likely represent cases where CMV reactivation occurred despite effective HIV suppression. Participants with no CMV DNA predominantly had high $CD4^+$ T cell counts (>500 cells/μL), which is consistent with effective immune reconstitution and efficacy of their ARTs (19). Conversely, those with intermediate (200–500 cells/μL) or low (<200 cells/μL) $CD4^+$ T cell counts may be at greater risk for CMV reactivation, where immune suppression correlated strongly with CMV replication (7,27).

Interestingly, all participants with detectable CMV DNA, whether at low or high Ct values, had high $CD4^+$ counts (>500 cells/μL) and this could reflect individuals in a phase of immune recovery, where ART has restored sufficient $CD4^+$ levels, however, CMV reactivation persists temporarily. This finding aligned with studies that highlighted the dynamic nature of immune recovery in HIV, where opportunistic infections such as CMV may still posed a threat even after initial immune reconstitution (28-30).

Finally, the moderately negative correlation (r<0) between CMV DNA Ct values and the age of participants suggests that CMV load increases as participants grew older, which highlights a possible cumulative effect of CMV exposure or reactivation over time. This aligned with findings from studies such as Slyker et al., (31) and Redpath et al., (32) where it was demonstrated that older children and adolescents with HIV often have a longer duration of immune dysregulation, increasing their susceptibility to CMV reactivation or higher viral loads. Also, studies in sub-Saharan Africa have noted that adolescents living with HIV often experienced higher rates of co-infections, reinforcing the need for age-specific approaches in addressing therapeutic options (8).

Conclusion:

Our study reported the prevalence and dynamics of CMV co-infection in children and adolescents living with HIV in Ibadan, Nigeria, emphasizing the complex interplay between demographic factors, viral loads, immune recovery by $CD4^+$ T cell count assessment. A CMV prevalence of 13.0% was observed with variable levels of replication and a significantly higher prevalence rates in males compared to females.

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Contributions of authors:

OEB and EOC conceived and designed the study; EOC, MMB, MEO and OLS collected and analyzed the samples; OEB wrote the initial draft, OEB, OLS, MMB, and MEO reviewed the final draft, while OEB, ECO and OLS supervised the entire research.

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Conflict of interest:

Authors declare no conflict of interest

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