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Original Research Article

Human cytomegalovirus infection, viraemia and retinitis among people living with HIV/AIDS in Kano, North-Western Nigeria

Chinagozi P. Edwin[1], Sadiq Hassan[3]*, Philips I. Ebisike[4], Saudat G. Habib[3], Taiwo G. Amole[5], Rasheed A. Bakare[2]

[1]Department of Clinical Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria
[2]Department of Clinical Microbiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[3]Department of Ophthalmology, College of Health Sciences, Bayero University/Aminu Kano Teaching Hospital, Kano, Nigeria
[4]Departmentof Optometry, College of Health Sciences, Bayero University/Aminu Kano Teaching Hospital, Kano, Nigeria
[5]Africa Centre of Excellence for Population Health and Policy, Bayero University/Aminu Kano teaching Hospital, Kano, Nigeria

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***Correspondence:**
Sadiq Hassan,
E-mail: sadiqh@yahoo.com

ABSTRACT

Background: Human cytomegalovirus (HCMV) is a leading cause of opportunistic infection in HIV-infected patients. HCMV viraemia is an active infection marker and prelude to end-organ diseases (EODs), such as retinitis. The aim of the study was to assess the burden and associated factors of HCMV infection, viraemia and retinitis among HIV-infected patients in Nigeria.
Methods: Comparative cross-sectional study of 160 HIV-infected adults, comprising 80 participants in each of <100/mm^3 and ≥100 cells/mm^3 CD4+ cell count groups, who attended HIV clinic at a tertiary hospital located in a major Nigerian city. A questionnaire was used to collect data from eligible consenting participants and their case files. Sera from all participants were tested for anti-HCMV IgG using ELISA method, and plasma of seropositive participants were subjected to PCR for HCMV viraemia. Participants whose samples were HCMV viraemic were examined for HCMV retinitis using indirect ophthalmoscopy. Data was analyzed using Minitab vs 14.1.1PP.
Results: All 160 participants tested positive for anti-HCMV IgG. HCMV viraemia was 14.4% (23 of 160) generally, but comparatively more among <100 CD4 cells/mm^3 group (18.8%; 15 of 80) than in ≥100 cells/mm^3 patient group (10%; 8 of 80). Only HCMV viraemic patients in <100 CD4 cells/mm^3 group (20%; 3 of 15) were diagnosed with HCMV retinitis. WHO stage was associated with HCMV viraemia (χ^2= 7.79, p=0.05) and HCMV retinitis (χ^2= 4.60, p=0.03). The only predictor of HCMV retinitis was WHO staging I and II [aOR = 0.04, 95%CI (0.01- 0.52)].
Conclusions: Evidence of previous and active HCMV infection is prevalent among PLWHA in Nigeria with WHO staging being associated and a predictor of HCMV viraemia and retinitis, respectively.

Keywords: Human cytomegalovirus, Human immunodeficiency virus, Kano, Retinitis, Viraemia

INTRODUCTION

Human cytomegalovirus (HCMV) is a herpesvirus that infects only humans, causing a highly prevalent latently persistent infection in immunocompetent adults, which makes it a leading cause of opportunistic infection in immunocompromised conditions, such as advanced Human immunodeficiency virus (HIV) infection.[1-5] By adulthood, most people in developing countries like Nigeria are said to have had primary HCMV infection,

with some studies reporting up to 100% prevalence of anti-HCMV IgG, a marker of previous infection, among immunocompetent individuals.[3,6-9]

Nigeria has one of the largest number of people living with HIV/AIDS (PLWHA).[10] Reactivation of latent HCMV infection in immunosuppressed individuals leads to viraemia, dissemination of the virus, and end-organ diseases (EODs), such as retinitis, colitis, pneumonitis and encephalitis.[1-5] HCMV viraemia is therefore regarded as the hallmark of active infection.[11] It reflects actively replicating HCMV infection and shedding of the virus into circulation.[12] Furthermore, HCMV has been found to synergistically act with HIV at the cellular level in potentiating its pathogenicity and progression to AIDS, thereby creating a vicious cycle of advancing immunodeficiency and progressing HCMV disease in co-infected persons.[1-3,13-15]

Although the advent of highly active antiretroviral therapy (HAART) has reduced the occurrence of HCMV EOD, reports show that HCMV remains a major cause of morbidity and mortality in patients with advanced HIV infection.[1,15-18] Low CD4+ cells count is a known marker of immunosuppression from HIV, and a major risk factor for the development of HCMV viraemia and EODs, whether or not a patient is on HAART.[11,14,16,17] HCMV retinitis, accounts for up to 80% of all HCMV EODs and remains the commonest cause of visual loss in people with AIDS.[1-3,11]

Early identification of predictors of active HCMV infection in immunosuppressed patients permits prompt commencement of treatment with anti-HCMV agents, thereby reducing the risk of development or progression of HCMV EOD in these patients.[4,14,19] Despite high prevalence of HIV and of HCMV infection in Nigeria, there are insufficient studies on their co-infection in the country. To the best of our knowledge at the time of writing this manuscript, among Nigerians living with HIV, only one study had looked at the prevalence of HCMV viraemia, while a few others have reported on HCMV retinitis using solely clinical examination.[20-22]

Although detection of certain features by indirect ophthalmoscopy is used in clinical diagnosis of HCMV retinitis, because these features are not completely pathognomonic, and clinical diagnosis can be subjective, molecular detection of HCMV viraemia helps to reinforce accuracy of diagnosis.[3,4,11] Reports have shown a wide variation in prevalence of HCMV viraemia and retinitis among HIV-infected patients across different populations.[23-26] Available studies, which so far have solely used clinical evaluation may have over-or under-reported the burden of HCMV retinitis in PLWHA in Nigeria.

The aim of the study was to determine prevalence of HCMV infection, HCMV viraemia, HCMV retinitis and their associated risk factors in PLWHA in Nigeria.

METHODS

Study design, setting and ethics

It was a six-months comparative cross-sectional study carried out at the HIV clinic of Aminu Kano Teaching Hospital (AKTH) Kano, a public tertiary hospital in Kano, a cosmopolitan Nigerian city, with over 20,000 registered HIV-positive patients, between September 2014 and May 2015.

All confirmed HIV-infected adults≥18 years of age, who consented to participate and had their CD4+ cells count measured within past six months were enrolled, but those with established ocular disease or features other than those typical of possible HCMV retinitis were excluded. An adapted questionnaire was used to collect information on socio-demographic characteristics, past history of blood transfusion and typical symptoms of HCMV retinitis. Information on CD4+ cell count, HAART status and WHO clinical stage were extracted from respondents' medical files. Follow-up ophthalmoscopy on HCMV viraemia-positive participants were initiated via telephone calls. Ethical approval was obtained from the Health Research and Ethics Committee of Aminu Kano Teaching Hospital. Only patients who gave informed consent to participate in this study were enrolled. All procedures were carried in line with WHO Helsinki declaration on human research.

Laboratory assessment

Blood samples of all 160 participants were divided into two aliquots, separated into plasma and sera respectively, and stored at -80^{0}C until participants' enrolment was completed. To avoid systematic and limit random errors, all participants sera were batch-screened for anti-HCMV IgG and IgM as markers of previous infection, using a qualitative sandwich third generation ELISA (Diagnostic Bioprobes Milan, Italy), according to the manufacturer's protocol, while plasma samples of all participants that tested positive for anti-HCMV IgG, were subjected to molecular testing for HCMV viraemia using conventional nested PCR technique. To carry out the PCR assay, genomic DNA was extracted and purified from plasma of all 160 participants, whose sera had tested positive for anti-HCMV IgG, using E.Z.N.A Blood DNA Mini Kit (Omega Bio-tek, Georgia, USA) according to manufacturer's instructions. The extracted DNA was immediately stored at -20^{0}C prior to PCR assay.

Using same materials and method of a previous study, the 1st round of a nested PCR was carried out to amplify a 309bp region in HCMV pp65 lower matrix phosphoprotein gene (UL 83 gene, accession number NC 001347), using a pair primer sequence: forward (HCMVF1)-5`TCACCTGCATCTTGGTTGCG 3`and reverse (HCMVR1)-5' TGCCGCTCAAGATGCTGAAC 3', which had been designed to amplify highly conserved regions of the gene while the second round was carried out

to amplify a 220 bp region within the first amplicon sequence, using a second pair of primers: forward (CMVF2)-5`-GGAAACACGAACGCTGACGT-3`and reverse (CMVR2)-5`-TGCCGCTCAAGATGCTGAAC-3'.[27] Bioneer Accu power Taq Hot Stat PCR premix (California, USA) was used to carry out PCR assay in Stratagene Robocycler Gradient 40 Thermal Cyler (California, USA) For quality control, a known positive and a negative sample were included during each amplification run. Post-amplification analysis of the gene products were done by electrophoresis in 1.5% agarose gels and subsequent photo-documentation using Bio-Rad Gel-Doc 2000 (California USA) connected to a computer set. Figure 1 shows gel electrophoresis on PCR products for HCMV DNA from specimens among the participants.

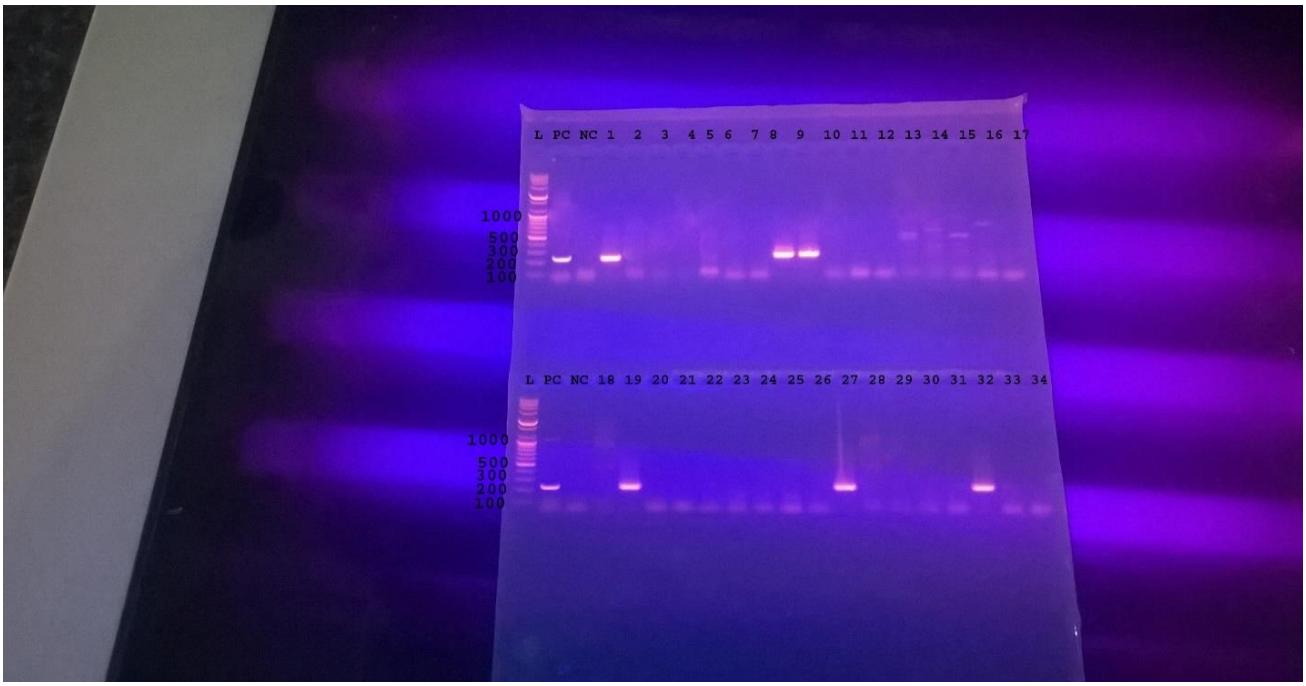

Figure 1: Gel electrophoresis on PCR product for HCMV DNA from specimens among the participants.

Note: L -DNA 'Ladder' (molecular weight marker) at 100 bp gradient, PC- positive control, NC- negative control. Positive samples: Lanes 1, 8, 9, 19, 27 and 32; and negative samples: Lanes 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 33, 34.

Clinical assessment

Patients who tested positive for HCMV viraemia were contacted for ophthalmoscopy via telephone calls. This was done by an experienced ophthalmologist, during their follow-up to the ART clinic. The HCMV viraemia status of the referred patients was not known to the ophthalmologist. Ophthalmoscopy was done using binocular indirect ophthalmoscope with 20-diopter lens through pupils fully dilated (using a combination of 5% phenylephrine and 0.8% tropicamide as reported in a previous study according to Centers for Disease Control (CDC) guideline on clinical diagnosis of HCMV retinitis.[11,28] According to established literature report, HCMV retinitis clinical diagnosis was made if the characteristic yellow to white, 'fluffy' to 'dry or granular' area(s) of full thickness retinal necrosis with or without haemorrhage and usually spreading centrifugally in a 'bushfire' fashion was observed.[3,11,28] Presence or absence of HCMV retinitis in either or both of participant's eyes was reported by the ophthalmologist. Figure 2 is a retinal examination image of one of the patients diagnosed of HCMV retinitis.

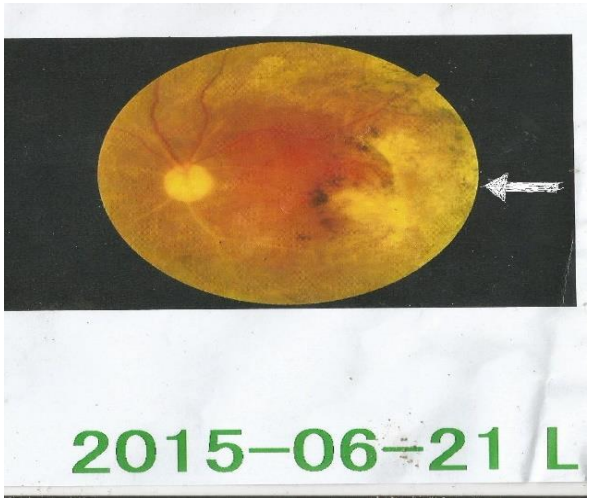

Figure 2: Retinal examination image of one the patients diagnosed of HCMV retinitis showing yellow white areas (pointed to by arrow) of retinal necrosis, that is characterized by dense retinal whitening surrounded by areas of varying fluffy to granular appearance and some haemorrhage.

Statistical analysis

Data were analysed using Minitab, version 14.1.1PP (Minitab Inc. State College, Pennsylvania USA) to describe participants' characteristics, sero-positivity, HCMV viraemia and HCMV retinitis, both separately in each study arm and cumulatively. Association of independent categorical variables (patient's socio-demographic and clinical characteristics) with outcome variables (HCMV viraemia and retinitis) were tested using Chi square test or Fisher's exact test as appropriate. A p value of ≤0.05 were considered statistically significant. Variables significantly associated with retinitis were then considered for logistic regression analysis to determine predictors of HCMV retinitis.

RESULTS

Socio-demographic characteristics

There were 80 patients with CD4 cell counts <100 cells/mm^3 and 80 patients with CD4 counts ≥100 cells/mm^3 recruited into the two arms of the study. Their ages ranged from 18 to 65 years with a mean of 39.9±12.6 years. There were 87 (54.4%) females and 73 (45.6) males giving a male to female ratio of 0.84. Among those with <100 cells/mm^3, CD4 cell count ranged from 6 to 97 with a mean of 48±5.7 cells/mm^3, while among those with CD4 cell count ≥100 cells/mm^3, it ranged from 102 to 900 with a mean of 331±69.5 cells/mm^3.

Among the <100 cell/mm^3 CD4 cell count group, there were about equal number of males (52.5%) and females (47.5%) while there were more females (61.2%) than males (38.8%) in the other arm. Majority (N=112;70%) of participants were Hausas by ethnicity and were of Islamic faith (N=126; 78.7%).

Most (80.6%) participants had at least basic primary education as opposed to 19.4% who had informal or no form of education. 52.5% among the <100 cells/mm^3 CD4+ count arm and 50.0% in the ≥100 cells/mm^3 arm) were married. Only one participant, who had CD4 count <100 cells/mm^3 volunteered to be an intravenous drug user (IVDU) but majority (N=159; 99.4%) of the participants were in the heterosexual risk group and in the WHO clinical stage I (N=130; 81.2%). Only 18.8% (N=30) had at least an episode of blood transfusion in the past (Table 1).

Prevalence of HCMV seropositivity and viraemia

Anti-HCMV IgG was positive in 100% (160 of 160) of participants. Therefore, plasma samples from all participants were subjected to PCR for HCMV viraemia, (14.4%; 23 of 160) tested positive.

HCMV viraemia among <100 CD4 cells/mm^3 participants group (15 of 80; 18.8%) was almost double that in ≥100 cells/mm^3 patient group (10%; 8 of 80) (Table 2).

Association of HCMV viraemia with socio-demographic characteristics and clinico-laboratory parameters of patients

The socio-demographic characteristics of respondents were not significantly associated with the occurrence of HCMV viraemia. Although not statistically significant, HCMV viraemia among <100 CD4 cells/mm^3 patient group was almost double that as opposed to among ≥100 CD4 cells/mm^3 group (18.8% vs 10.0%; χ^2=2.49; p=0.12).

HCMV viraemia among patients in advanced HIV stage (WHO stages III and IV) more than tripled that, in combined WHO stages I and II, demonstrating strong association between WHO stage and HCMV viraemia (38.9% vs 11.3%; χ^2=7.79; p=0.005). Although there was a lower prevalence of HCMV viraemia among patients who were not on HAART as compared to their counterparts on HAART, the difference was not statistically significant (12.8% vs 33.3%; χ^2=2.31; p=0.073). History of previous blood transfusion was not statistically significantly associated with HCMV viraemia among patients (Table 3).

Prevalence of HCMV retinitis among HCMV viraemia-positive patients

Out of the 15 HCMV viraemic patients in <100 CD4 cells/mm^3 group, 20% (3 of 15) were diagnosed with HCMV retinitis, while none of the 8 HCMV viraemic patients in the ≥100 cells/mm^3 group had retinitis. Cumulatively, the prevalence of HCMV retinitis among all 23 HCMV viraemic participants was 13.0%, and 1.9% among all 160 study participants. No statistically significant association was found between HCMV retinitis and age or sex.

Less than 1% of participants in combined WHO stages I and II group had HCMV retinitis while 11% in combined stages III and IV group, signifying an association between WHO stage and HCMV retinitis (χ^2=4.60; p=0.03). All the 3 patients diagnosed of HCMV retinitis had a history of at least one of the typical symptoms associated with HCMV retinitis (floaters, scotoma, photopsia, blurred vision or loss of vision). There was an association between HCMV retinitis-associated ocular symptoms and actual presence of HCMV retinitis (χ^2=5.99; p=0.01) (Table 4).

Logistic regression analysis for predictors of HCMV retinitis

WHO stages and ocular symptoms which were both found to have statistically significant association with HCMV retinitis were included in the logistic regression model to determine predictors for HCMV retinitis. WHO stage was found to be a predictor for HCMV retinitis.

Patients in WHO stages I and II were 96% less likely to have HCMV retinitis compared to those in stages III and IV [OR = 0.04, 95%CI (0.01- 0.52)], p=0.02 (Table 5).

Table 1: Frequency distribution of enrolled patients in both the <100 cell/mm^3 and ≥100 cell/mm^3 CD4 count arms based on their baseline characteristics.

Baseline characteristics	Frequency		
	CD4+ count <100 cells/mm^3, N=80, n (%)	CD4+ count ≥100 cell/mm^3, N=80, n (%)	Total, N=160, n (%)
Age group (years)			
Less than 30	16 (20.0)	10 (12.5)	26 (16.3)
30-39	33 (41.2)	32 (40.0)	65 (40.6)
40-49	22 (27.5)	22 (27.5)	44 (27.5)
≥50	9 (11.3)	16 (20.0)	25 (15.6)
Gender			
Male	42 (52.5)	31 (38.8)	73 (45.6)
Female	38 (47.5)	49 (61.2)	87 (54.4)
Tribe			
Hausa	58 (72.5)	54 (67.5)	112 (70)
Igbo	5 (6.2)	8 (10.0)	13 (8.1)
Yoruba	3 (3.8)	2 (2.5)	5 (3.1)
Others	14 (17.5)	16 (20.0)	30 (18.8)
Religion			
Christianity	12 (15.0)	22 (27.5)	34 (21.2)
Islam	68 (85.0)	58 (72.5)	126 (78.8)
Level of education			
Tertiary	13 (16.3)	18 (22.5)	31 (19.4)
Secondary	29 (36.2)	32 (40.0)	61 (38.1)
Primary	20 (25.0)	17 (17.2)	37 (23.1)
Informal/none	18 (22.5)	13 (16.3)	31 (19.4)
Marital status			
Single	15 (18.8)	15 (18.8)	30 (18.8)
Married	42 (52.5)	38 (47.5)	80 (50)
Divorced	5 (6.2)	4 (5.0)	9 (5.7)
Widowed	18 (22.5)	23 (28.7)	41 (25.6)
Risk groups			
IVDU	1 (1.3)	0(0.0)	1 (0.6)
MSM	0	0	0
CSW	0	0	0
Heterosexual	79 (98.7)	80 (100)	159 (99.4)
WHO clinical stage			
I	55 (68.7)	75 (93.7)	130 (81.2)
II	10 (12.5)	2 (2.5)	12 (7.5)
III	11 (13.8)	0	11 (6.9)
IV	4 (5.0)	3 (3.8)	7 (4.4)
HAART status			
On HAART	71 (88.7)	77 (96.2)	148 (92.5)
Not on HAART	9 (11.3)	3 (3.8)	12 (7.5)
Previous blood transfusion			
Yes	16 (20.0)	14 (17.5)	30 (18.8)
No	64 (80.0)	66 (82.5)	130 (81.2)

Note: IVDU= Intravenous drug use, MSM= Men who have sex with men, CSW= Commercial sex worker.

Table 2: Prevalence of HCMV viraemia and its association with variable baseline characteristics of study participants.

Variable characteristics	CMV viraemia		Total N=160, n (%)	χ^2	P value
	Positive N=23, n (%)	Negative N=137, n (%)			
Age (years)					
Less than 40	14 (13.1)	77 (77.9)	91 (100)	0.175	0.821
40 and above	9 (9.9)	60 (59.1)	69 (100)		

Continued.

Variable characteristics	CMV viraemia		Total N=160, n (%)	χ^2	P value
	Positive N=23, n (%)	Negative N=137, n (%)			
Gender					
Female	12 (13.8)	75 (86.2)	87 (100)	0.052	0.865
Male	11 (15.1)	62 (84.9)	73 (100)		
Tribe					
Hausa	20 (17.9)	92 (82.1)	112 (100)	2.795	0.083
Others	3 (6.3)	45 (93.7)	48 (100)		
Educational status					
Formal	18 (14.0)	111 (86.0)	129 (100)	0.096	0.77
Informal/none	5 (16.3)	26 (83.9)	31(100)		
Marital status					
Never married	5 (16.7)	25 (83.3)	30 (100)	0.158	0.691
Ever married	18 (13.9)	112 (86.1)	130 (100)		

Table 3: Prevalence of HCMV viraemia and its association with variable baseline clinico-laboratory parameters of participants.

Variable characteristics	HCMV viraemia		Total N=160, n (%)	χ^2	P value
	Positive N=23, n (%)	Negative N=137, n (%)			
CD4+ cell count (cells/mm^3)					
Less than 100	15 (18.8)	65 (81.2)	80 (100)	2.488	0.115
100 and above	8 (10.0)	72 (90.0)	80 (100)		
WHO stage					
Stage I or II	16 (11.3)	126 (88.7)	142 (100)		
Stage III or IV	7 (38.9)	11 (61.1)	18 (100)	7.79	0.005*
HAART status					
On HAART	19 (12.8)	129 (87.2)	148 (100)		
Not on HAART	4 (33.3)	8 (66.7)	12 (100)	2.306	0.073
History of previous transfusion					
Yes	4 (13.3)	26 (86.7)	30 (100)	0.012	0.914
No	19 (14.6)	111 (85.4)	130 (100)		

*Statistically significant (by Fisher's exact test).

Table 4: Overall prevalence of HCMV retinitis and its association with some socio-demographic baseline clinic-laboratory characteristics of study participants.

Variable characteristics	HCMV retinitis		Total N=160, n (%)	χ^2	P value
	Present N=3, n (%)	Not present N=157, n (%)			
Sex					
Female	3 (3.4)	84 (96.6)	87 (100)	1.334	0.309
Male	0	73 (100.0)	73 (100)		
Age (years)					
Less than 40	1 (2.9)	91 (98.9)	92 (100)	0.070	0.791
40 and above	2 (2.9)	66 (97.1)	68 (100)		
CD4+ cell count (cells/mm^3)					
Less than 100	3 (3.8)	77 (96.2)	80 (100)	1.359	0.244
100 and above	0	80 (100)	80 (100)		
WHO stage					
I or II	1 (0.7)	141 (99.3)	142 (100)		
III or IV	2 (11.1)	16 (88.9)	18 (100)	4.598	0.032*
Ocular symptom					
Present	3 (7.9)	35 (92.1)	38 (100)	5.994	0.014*
Not present	0	122 (100)	122 (100)		
HAART status					
On HAART	3 (2.0)	145 (98.0)	148 (100)	0.370	0.543
Not on HAART	0	12 (100)	12 (100)		

*Statistically significant (by Fisher's exact test).

Table 5: Bivariate logistic regression analysis of two selected variables to test their predictive value for the presence HCMV retinitis among 160 HIV-positive patients.

Predictor variables	Adjusted OR (95% CI)	P value
WHO stage		
I or II	0.04 (0.01-0.52)	0.02*
III or IV	Referent	
Ocular symptom		
Not present	Referent	0.07
Present	12.58 (0.85-186.85)	

Note: *Statistically significant; OR= odds ratio; CI= confidence interval.

DISCUSSION

In this study, anti-HCMV IgG was detected in all participants, which means a 100% prevalence of previous HCMV infection. Primary HCMV infection invariably leads to a latent persistent infection that can reactivate due to immunodeficiency to cause HCMV viraemia and EOD, most commonly retinitis in PLWHA. This result shows that most PLWHA in Nigeria have a potential risk of developing active HCMV infection and retinitis with a higher risk among patients at WHO stages III and IV, having <100 cells/µL CD4+ cell count, compared with those at WHO stages I and II or with higher CD4+ cell counts. Active HCMV infection, evidenced by HCMV viraemia, precedes HCMV retinitis which was ophthalmoscopically detected in 13% of all HCMV viraemic participants. Retinitis was not detected in any of the HCMV viraemic participants with CD4+ cell count ≥100 cells/µL. This may signify that retinitis is more likely to occur and should be sought in HCMV viraemic PLWHA at WHO stages III or IV with <100 cells/µL CD4+ cell count.

The 100% prevalence rate of anti-HCMV IgG positivity among all participants in this study is in keeping with a previous study by Akinbami et al, which also reported the same rate among HIV patients attending a tertiary hospital in another region of Nigeria.[7] This rate is a reflection of the rate in the general population. Among healthy individuals, Hamid et al reported anti-HCMV IgG positivity rate of 91.1% in pregnant women, while Gwarzo et al reported 100% among healthy blood donors, both in Kano-Nigeria.[29,30] These results supports an already existing knowledge, that most adults in regions of the developing world like Nigeria have had a previous HCMV infection.[3,6] This invariably puts them at risk of reactivated HCMV infection and subsequent EOD such as retinitis if they develop immunosuppression due, for instance, to HIV/AIDS.

The prevalence of HCMV viraemia, the marker of active HCMV infection, in our study (14.4%) was almost the same as a 14.8% rate reported from Lagos by Akinwale et al.[20] Our study, by finding a similar HCMV viaemia rate, reinforces the burden of active HCMV infection among PLWHA in Nigeria. This suggests that a significant number of PLWHA in Nigeria are at a potential risk of developing HCMV EOD, such as retinitis.

In this study HCMV retinitis was diagnosed by ophthalmoscopically identifying typical ocular features of HCMV retinitis in patients with HCMV viraemia. HCMV retinitis was detected in 3 out of the 23 participants in this study who tested positive for HCMV viraemia. Previous studies in Nigeria have reported rates of HCMV retinitis in PLWHA ranging from 0 to 6% compared to as high as 41.9% reported in the US.[21,22,26] Given that the burden of HCMV infection in Nigeria is higher than in the U.S, the reason behind this wide disparity is not yet established but has been hypothesized to be due to death of HIV/AIDS patients in African earlier than when HCMV retinitis would have manifested or diagnosed.[31] However a study by Hodge et al associated the differences in risk of HCMV retinitis to difference in HLA types in people of different origins.[32] Also some level of subjectivity involved in diagnosis of HCMV retinitis because of differences in examiners judgements may be responsible for significant differences between prevalence outcomes from different studies particularly those that are not supported by detection of HCMV viraemia, as was done in this study. All the previous Nigerian studies generally examined PLWHA for HCMV retinitis without first testing them for active HCMV infection which is evidenced by HCMV viraemia.

Variations in prevalence of HCMV retinitis based on CD4 count are in keeping with the expected difference in immune status between participants in the groups stratified by CD4+ cell count and WHO HIV/AIDS disease stage. T-lymphocytes are regarded as the most critical element of the immune system in suppressing HCMV infection. Patients deficient in cell-mediated immunity are at greatest risk for HCMV disease. WHO stages 1 or 2 represent milder level of immunodeficiency, while stages 3 to 4 represent advanced to severe immunodeficiency, with presence of opportunistic infections of which HCMV is a leading cause.[34] Previous studies have shown that low CD4 cell count in advanced HIV-infected patients, particularly counts less than 50 to 100 cells/mm^3, is a major risk factor for HCMV viraemia and EOD.[6,11,14-17] In this study, prevalence of HCMV viraemia among participants with CD4+ cell count <100 cells/mm^3 was about 2 folds higher than among those with CD4+ cell count >100 cells/mm^3. Further stratification of participants as shown in Figure 3 showed that the prevalence rate of HCMV viraemia among those with CD4+ cell count <50 cells/mm^3 was almost 3 folds higher than that among other CD4+ cell count groups

which was fairly similar among them after the 50 cells/mm^3 threshold. In this study, the median CD4+ cell count for participant groups of <100 cell and >100 cell were 48 and 293 respectively.

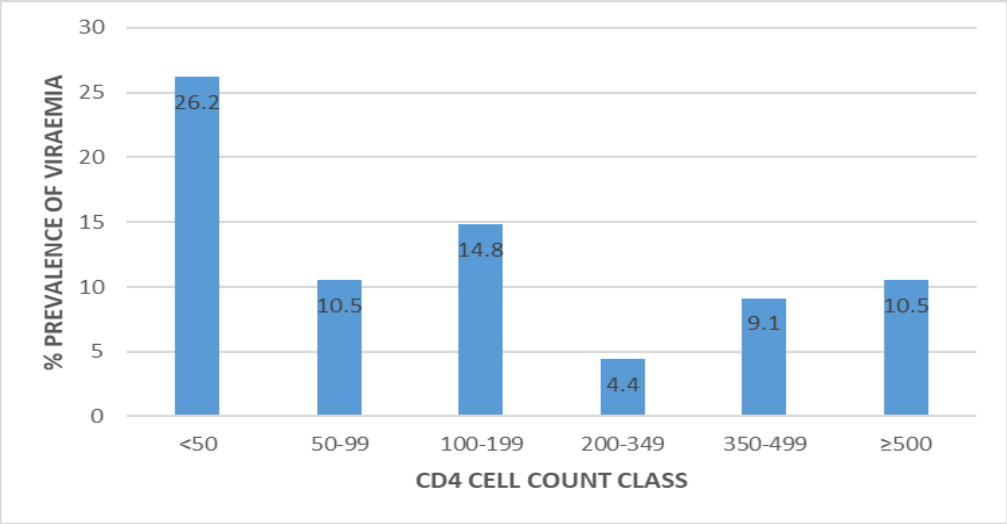

Figure 3: Prevalence of HCMV viraemia (PCR positivity) according CD4 count class.

In a study in South Africa, Fielding et al found that HCMV viraemia has a significant association with CD4+ cell count, whereby the group with a lower median value of CD4+ cell count had a greater proportion of HCMV viraemia compared to that with higher median value of CD4+ cell count and the proportion of HCMV viraemia found was higher in the study participants with CD4+ cell count of less than 100 cells/mm^3 compared to those with CD4+ cell count of greater than or equal to 100 cells/mm^3.[13]

In our study, the prevalence of HCMV viraemia among participants at WHO stages 3 or 4 was almost 4 folds that among those at stages 1 or 2. Although with less wide disparity, Fielding et al also found a higher proportion (43%) of HCMV viraemia in participants who were at WHO stage 3 or 4 compared to a 30% found among those who were at stage 1 or 2.[13] The proportion of HCMV viraemia was higher (33.3%) among participants who were not on HAART compared with those (12.8%) who were on HAART. This is expected since CD4+ cell count recovers with commencement of HAART leading to the recovery of cell-mediated immunity which suppresses HCMV replication. The suppressive effect of HAART on HCMV viraemia in the absence of anti-CMV-specific therapy has been demonstrated by Deayton et al.[35] We did not find any statistically significant association between HCMV viraemia and age, gender, educational status, marital status and history of previous blood transfusion.

Similarly, Akinwale et al also found no association between sex, marital status and HCMV viraemia, however, their study reported a marginally significant association between age and HCMV viraemia.[20] This difference is likely a function of the sampling technique, sample age distribution and age stratification in data analysis. Although possibly limited by sample size, proportionally, the prevalence of HCMV retinitis was more than 10 folds higher among WHO stages 3 or 4 group compared with stages 1 or 2 group. In a study by Niworth et al, that also stratified participants into two categories of WHO staging, the only two participants that were found to have HCMV retinitis were both in WHO stage category 'stage 3 or 4'.[28] All the participants diagnosed of HCMV viraemia had a history of one or more of floaters, scotoma, photopsia, blurred vision or loss of vision.

There were some limitations with our study. We detected HCMV retinitis in only 3 participants, and this may have been accounted by the fact that our study was hospital out-patient-based and hence looked at only those who were healthy enough to attend regular clinic follow-up, but HCMV retinitis is more likely to present in very ill patients at advanced stage of the disease. Although indirect ophthalmoscopy for HCMV retinitis on only participants that tested positive for HCMV viraemia, but because of the very low viral DNA detection limit (0.2 copies/ul) and high sensitivity of our study PCR protocol it is very unlikely that we missed any cases of HCMV viraemia or retinitis among our study participants.

However, to the best of our knowledge, this study is the only one that had ever examined HCMV viraemia in PLWHA in Nigeria. It has evaluated HCMV retinitis among HCMV viraemic PLWHA and highlighted the importance of a systematic HCMV retinitis case-finding, more so as HCMV retinitis is usually initially asymptomatic.

CONCLUSION

Although the cases of HCMV retinitis detected in our study were few, the high prevalence of both previous and active infection, evidenced by HCMV viraemia, which is

the prelude of HCMV retinitis show potential high risk of HCMV retinitis among our study population. Our finding of strong associations between CD4+ cell count, WHO stage and HCMV viraemia and retinitis is in agreement with existing knowledge but reinforces the need for clinicians practicing in our environment to use these as guides to determine patients that may require investigation for HCMV viraemia and retinitis, or their HIV management optimization to prevent occurrence of these.

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