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Seroprevalence of Cytomegalovirus among HIV-Infected Adult Patients on HAART

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CKO, CO, EIK and EOE were involved in the concept design, drawing up of protocol, collection and analysis of specimen. Authors CKO and VUN were involved in literature search, statistical analysis and drafting of the manuscript. All the authors read and approved the final manuscript.

Research Article

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ABSTRACT

Background/Aims: Cytomegalovirus is one of the opportunistic infections associated with significantly high morbidity and mortality among patients living with HIV/AIDS. Clinical manifestation of CMV infection is worse among HIV patients not receiving highly active antiretroviral therapy (HAART) than those receiving HAART. This study is aimed at investigating the seroprevalence of CMV among HIV-infected adults on HAART.
Study Design: This is a cross sectional study.
Place and Duration of Study: HIV Outpatient Clinic of University of Benin Teaching Hospital between April and September, 2011.
Methodology: A total of 342 HIV infected adult patients on HAART attending the HIV Outpatients Clinic of the University of Benin Teaching Hospital between April and September 2011, were screened for CMV IgG and IgM using ELISA method. Clinical

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stage of HIV disease and CD4+ cell counts were also evaluated. Data was analyzed using SPSS version 17.
Results: Of the 342 patients studied 338 (98.8%) were sero-positive for CMV IgG antibodies while 24 (7.0%) demonstrated sero-positivity for CMV IgM antibodies. The seroprevalence of CMV IgM was significantly higher in patients in WHO clinical stages 3-4 (10.9%) than those in stages 1-2 (4.7%) ($p = 0.04$). There is no significant statistical difference in sereoprevalences of IgG and IgM with respect to sex, age, and CD4+ cell counts.
Conclusion: Clinical stage of HIV/AID is an important risk factor for reactivation or re-infection of CMV and may prompt early diagnosis and management of active CMV infection.

Keywords: Cytomegalovirus; HIV; seroprevalence; HAART.

1. INTRODUCTION

Cytomegalovirus (CMV), a beta-herpesvirus, is a common opportunistic infection among human immunodeficiency virus (HIV)-infected individuals. Like other herpesviruses, this virus has a natural ability to enter latency after asymptomatic or mild symptomatic primary infection in immunocompetent hosts. In immunocompromised settings such as HIV/AIDS, the virus undergoes periodic episodes of reactivation [1,2]. Also immunocompromised hosts could also suffer CMV disease when they are re-infected by different exogenous strains of CMV virus [3]. Cytomegalovirus disease is associated with significantly high morbidity and mortality in the immunocompromised hosts [4]. Clinical manifestations of cytomegalovirus such as chorioretinitis, esophagitis, colitis, pneumonitis, adrenalitis and neurological disorders, have been observed in up to 40% of HIV-infected patients that are not on highly active antiretroviral therapy (HAART) [5]. Their counterparts who are on HAART tend to have lower incidence of CMV disease [6].

Primary infection leaves the sufferer with CMV-IgG seropositivity. The seroprevalence varies greatly with a variety of epidemiological factors such as age, geographical distribution, socioeconomic status, marital status and parity [7]. In healthy individuals in the various parts of the world, CMV-IgG seroprevalence ranges from 40-100% [8,9]. Among HIV/AIDS patients, seroprevalences of IgG and IgM ranges from 59.2-100.0% and 0.0-42.9% respectively [5,10,11].

There are reports of a synergistic interaction between HIV and CMV which is capable of worsening the immunologic profile and lead to more rapid progression to AIDS in HIV infected patient [7,12]. Also CMV may predispose the host to bacterial or fungal infection by compromising the integrity of mucosal barriers to infection [13]. On the other hand tumour necrotic factor (TNF) –α stimulated host cells accumulates intranuclear kB factor leading to activation of CMV DNA replication [7]

This study is aimed at investigating the seroprevalence of CMV among HIV-infected adults on HAART.

2. METHODOLOGY

2.1 Study Design, Population and Setting

This is a cross sectional study involving 342 HIV infected adult patients on highly active antiretroviral therapy (HAART) attending the HIV Outpatients Clinic of the University of Benin Teaching Hospital between April and September 2011. This is a 700 bed tertiary hospital located and serving as a major referral centre in the southern part of Nigeria. Informed consents were sought and consecutively consenting patients were recruited into the study. Biodata and clinical histories were obtained through interviewer-administered structured questionnaires and patients' case notes. For the purpose of this study participants were categorized into asymptomatic (stage 1 and 2) and symptomatic (stage 3 and 4) in line with WHO clinical staging system [14].

2.2 Laboratory Methods

The HIV status of participants was already determined through their plasma samples screened with two different methods namely; Abbott determine HIV 1 & 2 kit which is an *in vitro* visually read immunoassay (Abbot Japan C. Ltd. Tokyo, Japan) and HIV 1 & 2 STAT-PAK Assay kit, which is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2 in human plasma (CHEMBIO Diagnostic system, INC, New York, USA). These tests were carried out strictly following the manufactures' instructions. Both methods have inherent quality controls that were used to validate results. Seropositivity for the two methods was used to classify patients as HIV infected.

Five millilitres of blood was drawn from each HIV infected patient and shared into a sterile unanticoagulated and an EDTA bottles. The former was centrifuged, sera separated and stored at -20ºC and later analysed for CMV IgG and IgM while the later was used for CD4+ cell count. Clinotech CMV IgG and IgM ELISA test kits (Canada) were used in studying sera CMV IgG and IgM antibodies respectively following the manufacturer's instruction. In summary, the patients' sera were diluted according to the manufacturer's instructions and added to wells already coated with purified CMV-Ag. It is expected that after a while, the antibodies if present would bind to the antigen. All unbound materials are washed away using the washing buffer. The enzyme conjugate was then added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the colour generated is proportional to the amount of antibody in the sample. Optical density (OD) ratio range is as follows: ≤ 0.90 for negative specimen, ≥ 1.00 for positive specimen and 0.91-0.99 for equivocal specimen. The patients CD4+ cell counts were determined using flow cytometry.

2.3 Statistical Analysis

Data were analysed using SPSS version 17. Descriptive data was presented as mean± standard deviation (SD). Chi square or Fisher's exact was used where appropriate to test association. Statistical significance was presumed where $p < 0.05$.

3. RESULTS AND DISCUSSIONS

A total of 342 HIV infected patients participated in this sero-survey carried out in the HIV Outpatients Clinic of the University of Benin Teaching Hospital between April and September 2011. This comprised of 90 (26.3%) males and 252 (73.7%) females giving a male-to-female

ratio of 1:2.8. Their ages ranged between 19 and 69 with a mean age of 40±10 years. Majority of the patients studied (39.2%) were in the 31-40 years age bracket while only 1.5% fell above 60 years (Table 1). Following the WHO clinical staging system, 214 (62.6%) were in stages 1 and 2 while the remaining 128 (37.4%) were in stages 3 and 4. About half (50.8%) of the patients studied had CD4+ cell counts ≤200/µl (Table 1).

Table 1. General characteristics of participants

Characteristics	Frequency	Percentage
Gender		
Male	90	26.3
Female	252	73.7
Age Group (yrs)		
≤20	6	1.8
21-30	49	14.3
31-40	134	39.2
41-50	89	26.0
51-60	59	17.3
>60	5	1.5
Disease Stage (WHO)		
1-2	214	62.6
3-4	128	37.4
CD4+Cell Count (/µl)		
≤200	174	50.8
>200	168	49.2

Of the 342 patients studied, 338 (98.8%) were sero-positive for CMV IgG antibodies while 24 (7.0%) demonstrated sero-positivity for CMV IgM antibodies (Table 2).

Table 2. Seroprevalence of cytomegalovirus among participants (n=342)

CMV antibodies	Number tested	Number positive (%)	Number negative (%)
IgG	342	338 (98.8)	4 (1.2)
IgM	342	24 (7.0)	318 (93.0)

Seroprevalence of CMV IgG antibody was slightly more in the female patients (99.2%) than males (97.8%) but this was not statistically significant ($p = 0.28$) as shown in Table 3. Also seroprevalence of CMV IgG antibody was found to increase with age from 66.7% among patients less than 20 years to 100.0% among patient that are 41 years and above (Table 3). Patients presenting at stages 1-2 of WHO clinical staging had a non-significant lower CMV IgG seroprevalence of 98.6% than those in stages 3-4 (99.2%) ($p = 0.32$), while an equal seroprevalence of 98.9% was recorded for both patients with CD4+ cell counts of ≤200**/**µl and >200 **/**µl respectively (Table 3).

Table 3. IgG-CMV status of participants

Characteristics	IgG positive (%)	IgG negative (%)	Total	p-value	Odds ratio
Gender					
Male	88 (97.8)	2 (2.2)	90	0.28	0.97
Female	250 (99.2)	2 (0.8)	252		
Age Group (yrs)					
≤20	4 (66.7)	2 (33.3)	6		
21-30	48 (98.0)	1 (2.0)	49		
31-40	133 (99.2)	1 (0.8)	134	0.33	
41-50	89 (100.0)	0 (0.0)	89		
51-60	59 (100.0)	0 (0.0)	59		
>60	5 (100.0)	0 (0.0)	5		
Disease stage (WHO)					
1-2	211 (98.6)	3 (1.4)	214	0.32	0.99
3-4	127 (99.2)	1 (0.8)	128		
CD4+Cell Count (/µl)					
≤200	172 (98.9)	2(1.1)	174	0.68	1.00
>200	166 (98.8)	2(1.2)	168		

On the other hand, for CMV IgM antibody sero-status, 7.1% of female patients studied were positive against 6.7% recorded by the male patients. Again this slight female preponderance is statistically not significant (*p = 0.88*) as illustrated in Table 4. Table 4 also shows that CMV IgM seropositivity did not vary with age in any particular direction as CMV IgG sero-status. Highest CMV IgM seroprevalence (20.0%) was recorded among patient greater than 60 years, followed by 8.2% recorded by 31-40 years age category, while those less than 20 years recorded zero percent seroprevalence. Also patients in WHO clinical stages 3-4 had a statistically significant higher CMV IgM seroprevalence of 10.9% than 4.7% observed in stages 1-2 (*p = 0.04*). Higher IgM seroprevalence (9.2%) was equally recorded for patients having CD4+ cell count ≤200**/**µl compared to 4.8% recorded among those with >200 **/**µl (*p = 0.67*).

The sero-survey of CMV antibodies among HIV-infected adult patients on HARRTs revealed that 98.8% of patients studied were seropositive for CMV IgG antibody. This figure is higher than 90.3% and 94.6% recorded in two separate studies among US women infected with HIV [15,16] and 95.3% among HIV-1 infected Adult patients in Botswana [17], but lower than 100.0% recorded in Iran [18]. Earlier study in Lagos, Nigeria recorded seroprevalence of 100.0% [19]. There is however a study in Ghana that reported an unusually low CMV IgG antibody seroprevalence of 59.9% among HIV infected patients [10]. High CMV IgG seroprevalence obtained in this study indicates that our patients harbour CMV virus in the latent form in their leucocytes and thus stand high risk of reactivation. They may therefore benefit from CMV prophylaxis with Ganciclovir to reduce incidence of active disease that may result from reactivation, especially those with CD4+ cell count lower than 50**/**µl [20].

Table 4. IgM-CMV status of participants

Characteristics	IgM positive (%)	IgM negative (%)	Total	p-value	Odds ratio
Gender					
Male	6 (6.7)	84 (93.3)	90	0.88	0.88
Female	18 (7.1)	234 (92.9)	252		
Age Group (yrs)					
≤20	0 (0.0)	6 (100.0)	6		
21-30	3 (6.1)	46 (93.9)	49		
31-40	11 (8.2)	123 (91.8)	134		
41-50	5 (5.6)	84 (94.4)	89	0.79	
51-60	4 (6.8)	55 (93.2)	59		
>60	1 (20.0)	4 (80.0)	5		
Disease Stage (WHO)					
1-2	10 (4.7)	204 (95.3)	214	0.04	0.16
3-4	14 (10.9)	115 (89.8)	128		
CD4+Cell Count (/µl)					
≤200	16 (9.2)	158 (90.8)	174	0.67	4.14
>200	8 (4.8)	160 (95.2)	168		

This study also revealed that CMV IgM antibody seroprevalence was 7.0%, a figure lower than 22.0% reported among HIV-1 positive homosexual men [21] and 9% reported among HIV infected Thai children [22], but higher than 2.3% recorded in USA [11]. An Iranian study reported CMV IgM seroprevalence of 42.9%, 0.0% and 0.8% among AIDS, HIV infected patients and 'healthy' controls respectively [23]. The variation in seroprevalence of CMV IgM observed may have to do with epidemiological and methodological differences. Also, seroprevalence of 7.0% indicates that a sizeable population of HIV infected patients in our environment has active CMV infection. Further study to determine whether these active infections are majorly due to reactivation or re-infection is advocated as this will impact so much on the management HIV infected patients. If active disease, for example, were to be mainly due to re-infection, the focus will be more on how to prevent exposure of HIV infected patients to possible sources of CMV infection such as donated blood/organs, respiratory droplets, saliva or urine from CMV infected patients [9]. If however reactivation is the major route to active CMV disease in HIV patients, then effort will be more on sustaining the patients' immune status.

Furthermore, seroprevalence of 7.0% for CMV IgM observed in this study is relatively high when compared with values reported among HIV negative populations. Figures as low as 0.171% was reported among healthy blood donors in India [24], 0.18% among pregnant women in Turkey [25], 2.3% among Brazilian blood donors [26] and 3% among a national representative population in US [27].This high prevalence may be due the reactivations and re-infections expected more among HIV infected patients given their compromised immune status. However, figures as high as 11% and 12% have been reported in India among pregnant women with bad obstetrics history and patients of paediatric age group respectively [28].

Seroprevalence of CMV IgG was slightly more in females than male in this study (99.2% versus 97.8%), but this was not statistically significant. This is consistent with other studies [17]. Preponderance of female could be explained by the fact that they probably have more

contact with babies as saliva and urine of infected children are significant sources of CMV infection [29]. The increase in CMV IgG seropositivity with age observed in this study agrees with the findings made in a similar study in Iran [5]. In this study there is no significant difference in seroprevalence of CMV IgG antibody with respect to clinical stage and CD4+ level. Similar finding was made also made (pls delete made) by Mehrkhani et al. [5]. The reason for this is not clear but since CMV is ubiquitous in this part of the world, most of our patients may have encountered this virus early in life and even before becoming infected with HIV. It is important to note that risk factors analysis for CMV IgG was hampered by the fact that nearly every patient was positive (98.8%) resulting in lack of power to detect risk factor for CMV IgG.

Another important observation made in this study is that seroprevalence of CMV IgM was significantly higher in the symptomatic clinical stages 3-4 (10.9%) compared to asymptomatic clinical stages 1-2 (4.7%) ($p = 0.04$). This is expected as the immune status of patients in the former group is likely more compromised than the later group, making them more susceptible to both reactivation and re-infection. Similarly seroprevalence of CMV IgM was also higher among patients with CD4+ cell count ≤ 200 */*µl (9.2%) compared to those >200 */*µl (4.8%) but this is not statistically significant ($p = 0.67$). Mehrkhani et al. [5] in their own study also observed no significant association between seroprevalence of CMV IgM and CD4+ level. The reason for this is not clear but since the patients are all on HAART which affects CD4+ cell counts, factors like duration and type of therapy may acts as cofounders. No relationship was established between CMV IgM seropositivity and gender and age.

4. CONCLUSIONS

Seroprevalences of CMV IgG and IgM among HIV infected adult patients on HAART were 98.8% and 7.0% respectively. Clinical stage of HIV/AID was an important risk factor for reactivation or re-infection of CMV and may prompt early diagnosis and management of active CMV infection. The result will be reduction in morbidity and mortality associated with CMV-HIV co-infection.

CONSENT

All authors have declared that written informed consents were obtained from the patients for publication of this work.

ETHICAL APPROVAL

This study was backed by ethical approval obtained from the Ethical committee of the University of Benin Teaching Hospital.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rawlinson W, Scott G. Cytomegalovirus: a common virus causing serious disease. Aust. Fam. Physician. 2003;32:789-93.
2. Feng-Qin F, Qi-Shi F, Zhi-Jun Y, Yi-Bing P, Li Z, Ke-Zi M. Incidence of Cytomegalovirus Infection in Shanghai, China. Clin Vaccine Immunol. 2009;16(11):1700–03.
3. Drew WL, Sweet ES, Miner RC, Mocarski ES. Multiple infections by Cytomegalovirus in patients with acquired immunodeficiency syndrome: documentation by Southern blot hybridization. J Infect Dis. 1984;150:952-53.
4. Springer KL, Weinberg A. Cytomegalovirus infection in the era of HAART: fewer reactivations and more immunity. Journal of Antimicrobial Chemotherapy 2004;54:582–86.
5. Mehrkhani F, Jam S, Sabzvari D, Fattahi F, Kourorian Z, SeyedAlinaghi S. Cytomegalovirus Co-Infection in Patients with Human Immunodeficiency Virus in Iran.2011 Acta Medica Iranica. 49;8:551-55.
6. Kempen JH, Jabs DA, Wilson LA, Dunn JP, West SK, Tonascia J. Mortality risk for patients withcytomegalovirus retinitis and acquired immune deficiency syndrome. Clin Infect Dis. 2003;37(10):1365-73.
7. Basawaraju A, Mane PM, Vijayadurga S. The Reactivation of the Cytomegalovirus (CMV) Infection in HIV Infected Patients. Journal of Clinical and Diagnostic Research, 2011;5(4):749-51.
8. Sinclair J, Sissons P. Latent and persistent infections of monocytes and macrophages. Intervirology. 1996;39 (5-6):293-301.
9. Ojide CK, E. Ophori EA, Eghafona NO, Omoti C. Seroprevalence of Cytomegalovirus (CMV) amongst voluntary blood donors In University of Benin Teaching Hospital (UBTH), Edo State, Nigeria. British Journal of Medicine & Medical Research. 2012;2(1):15-20.
10. Adjei AA, Armah HB, Gbagbo F, Boamah I, Adu-Gyamfi C, Asare I. Seroprevalence of HHV-8, CMV and EBV among the general population in Ghana, West Africa. BMC Infectious Disease. 2008;8:8-11.
11. Schoenfisch AL, Dollard SC, Amin M, Gardner LI, Klein RS, Mayer K, et al. Cytomegalovirus (CMV) shedding is highly correlated with markers of immunosuppression in CMV-seropositive women. Journal of Medical Microbiology. 2011;60:768–774.
12. Chakravarti A, Kashyap B, Matlani M. Cytomegalovirus infections: An Indian Perspective. Indian J Med Microbiol. 2009;27:3-11.
13. Schooley RT. Cytomegalovirus in the setting of infection with human immunodeficiency virus. Rev Infect Dis. 1990;12(7):811-9.
14. World Health Organization. WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children; 2007.
15. 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.

16. Stover CT, Smith DK,D. Scott Schmid D, Pellett PE, Stewart JA, Klein RS, et al. Prevalence of and Risk Factors for Viral Infections among Human Immunodeficiency Virus (HIV)–Infected and High-Risk HIV-Uninfected Women J Infect Dis. 2003;187:1388–96
17. Wester CW, Bussmann H, Moyo S, Avalos A, Gaolathe T, Ndwapi N, Essex M, MacGregor RR, Marlink RG. Serological Evidence of HIV-Associated Infection among HIV-1–Infected Adults in Botswana. Clin Infect Dis. 2006;43:1612–5
18. Abdollahi A, Shoar S, Sheikhbahaei S, Jafari S. Sero-prevalence of *Cytomegalovirus* and *Toxoplasma* Infections among Newly Diagnosed HIV Patients in Iran; Assessing the Correlation with CD4+ Cell Counts. Iranian Journal of Pathology. 2013;8(2):81–88.
19. Akinbami AA, Akanmu AS, Adeyemo TA, Wright KO, Dada MO, Dosunmu AO. Cytomegalovirus Antibodies amongst Immunocompromised (HIV) Patients at Lagos University Teaching Hospital (LUTH) Idi -Araba, Lagos. J Med. 2010;11:151-154.
20. Wingard JR, Piantadosis S, Burn WH. Pathogenesis of Cytomegalovirus infection after marrow transplantation. Infect Dis. 1999;153:478-88.
21. Leach CT, Cherry JD, English PA, Hennessey K, Giorgi JV, Visscher BR, Dudley JP, Detels R.The relationship between T-cell levels and CMV infection in asymptomatic HIV-1 antibody-positive homosexual men. J Acquir Immune Defic Syndr. 1993;6(4):407-13.
22. Likitnukul S, Bhattarakosl P and Poovorawan Y. Seroprevalence of Cytomegalovirus Infection in Children Born to HIV-1 Infected Women. 2003;21:127-130.
23. Orer AR Alizi SMH Al-Diwan JK Al-Hadithi TS. CMV infection among HIV / AIDS patients in Iraq. J Fac Med Baghdad. 2006;46(4):407-9.
24. Kumar H, Gupta PK, Kumar S, Sarkar RS. Is seroprevalence of anti-IgM CMV among blood donors relevant in India? Indian J Pathol Micobiol. 2008;51:351-2.
25. Uysal A, Taner CE, Cuce M, Atalay S, Gol B, Kose S. Uysal F. Cytomegalovirus and Rubella seroprevalence in pregnant women in Izmir/Turkey: follow-up and result of pregnancy outcome. Arch Gynecol Obstet. 2012;286:605-608.
26. Souza MA. Passos AM, Treitinger A, Spada C. Seroprevalence of Cytomegalovirus antibodies in blood donors in Southern, Brazil. Rev Soc Bras Med Trop. 2010;43:359-61.
27. Dollard SC, Staras SAS, Amin MM, Schmid DS and Cannon MJ. National Prevalence Estimates for Cytomegalovirus IgM and IgG Avidity and Association between High IgM Antibody Titre and Low IgG Avidity. Clin Vaccine Immunol. 2011;18:1895-1899.
28. Das S, Ramachandran VG, Arora R. Cytomegalovirus and Rubella infection in children and pregnant mothers- a hospital based study. J Commun Dis. 2007;39:113-117.
29. Lazzarotto T, Guerra B, Gabrielli L, Lanari M, Landini MP. Update on the prevention, diagnosis and management of cytomegalovirus infection during pregnancy. Clin Microbiol Infect. 2011;17:1285–1293.

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