

RESEARCH PAPER

**SEROPREVALENCE OF
CYTOMEGALOVIRUS AMONG HIV-
INFECTED ADULT PATIENTS ON
HAART.**

BY

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29 **ABSTRACT**

30 **Background/Aims:** Cytomegalovirus is one of the opportunistic infections associated with
31 significantly high morbidity and mortality among patients living with HIV/AIDS. Clinical manifestation of
32 CMV infection is worse among HIV patients not receiving highly active antiretroviral therapy (HAART)
33 than those receiving HAART. This study is aimed at investigating the seroprevalence of CMV among
34 HIV-infected adults on HAART.

35 **Study design:** This is a cross sectional study.

36 **Place and Duration of Study:** HIV Outpatient Clinic of University of Benin Teaching Hospital
37 between April and September, 2011.

38 **Methodology:** A total of 342 HIV infected adult patients on HAART attending the HIV Outpatients
39 Clinic of the University of Benin Teaching Hospital between April and September 2011, were
40 screened for CMV IgG and IgM using ELISA method. Clinical stage of HIV disease and CD4+ cell
41 counts were also evaluated. Data was analyzed using SPSS version 17.

42 **Results:** Of the 342 patients studied 338 (98.8%) were sero-positive for CMV IgG antibodies while 24
43 (7.0%) demonstrated sero-positivity for CMV IgM antibodies. The seroprevalence of CMV IgM was
44 significantly higher in patients in WHO clinical stages 3-4 (10.9%) than those in stages 1-2 (4.7%) ($p =$
45 0.04). There is no significant statistical difference in seroprevalences of IgG and IgM with respect to
46 sex, age, and CD4+ cell counts.

47 **Conclusion:** Clinical stage of HIV/AIDS is an important risk factor for reactivation or re-infection of
48 CMV and may prompt early diagnosis and management of active CMV infection.

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50 **Key words:** Cytomegalovirus, HIV, Seroprevalence, HAART.

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54 INTRODUCTION

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

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

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

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

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81 **METHODOLOGY**

82 **Study design, population and setting.**

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

91 **Laboratory Methods**

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

99 Five millilitres of blood was drawn from each HIV infected patient and shared into a sterile
 100 unanticoagulated and an EDTA bottles. The former was centrifuged, sera separated and stored at -20
 101 °C and later analysed for CMV IgG and IgM while the later was used for CD4+ cell count. Clinotech
 102 CMV IgG and IgM ELISA test kits (Canada) were used in studying sera CMV IgG and IgM antibodies
 103 respectively following the manufacturer's instruction. In summary, the patients' sera were diluted
 104 according to the manufacturer's instructions and added to wells already coated with purified CMV-Ag.
 105 It is expected that after a while, the antibodies if present would bind to the antigen. All unbound
 106 materials are washed away using the washing buffer. The enzyme conjugate was then added. The
 107 plate was incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the colour
 108 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.

Statistical analysis

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

RESULTS

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 $\leq 200/\mu\text{l}$ (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
2-4	128	37.4
CD4+Cell Count ($/\mu\text{l}$)		
≤ 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 $\leq 200/\mu\text{l}$ and $>200/\mu\text{l}$ respectively (Table 3).

Table 3: IgG-CMV Status of Participants

Characteristics	IgG positive (%)	IgG negative (%)	Total	p-value
Gender				
Male	88 (97.8)	2 (2.2)	90	0.28
Female	250 (99.2)	2 (0.8)	252	
Age Group (yrs)				
≤ 20	4 (66.7)	2 (33.3)	6	0.33
21-30	48 (98.0)	1 (2.0)	49	
31-40	133 (99.2)	1 (0.8)	134	
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
2-4	127 (99.2)	1 (0.8)	128	
CD4+Cell Count (μl)				
≤ 200	172 (98.9)	2(1.1)	174	0.68
>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 $\leq 200/\mu\text{l}$ compared to 4.8% recorded among those with $>200/\mu\text{l}$ ($p = 0.67$).

Table 4: IgM-CMV Status of Participants

Characteristics	IgM positive (%)	IgM negative (%)	Total	p-value
Gender				
Male	6 (6.7)	84 (93.3)	90	0.88
Female	18 (7.1)	234 (92.9)	252	
Age Group (yrs)				
≤ 20	0 (0.0)	6 (100.0)	6	0.79
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	
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
2-4	14 (10.9)	115 (89.8)	128	
CD4+Cell Count (μl)				
≤ 200	16 (9.2)	158 (90.8)	174	0.67
>200	8 (4.8)	160 (95.2)	168	

DISCUSSIONS

The sero-survey of CMV antibodies among HIV-infected adult patients on HARTs revealed that 98.8% of patients studied were seropositive for CMV IgG antibody. This figure is higher than 90.3% recorded among US urban minority HIV infected women [15] and 95.3% among HIV-1 infected Adult patients in Botswana [16], but lower than 100.0% recorded both in Iran [17]. Earlier study in Lagos, Nigeria recorded seroprevalence of 100.0% [18]. 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 $50/\mu\text{l}$ [19].

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 [20] and 9% reported among HIV infected Thai children [21], 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 [22]. 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.

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 is thought to be probably related to sexual transmission. 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 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.

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 / μ l (9.2%) compared to those >200 / μ l (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 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.

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/AIDS 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.

195 **ETHICAL APPROVAL**

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

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202 **COMPETING INTERESTS**

203 Authors have declared that no competing interests exist.

204 **AUTHORS' CONTRIBUTIONS**

205 Drs Ojide CK, Omoti C, Kalu EI and Ephraim OE were involved in the concept design, drawing up of
206 protocol, collection and analysis of specimen. Drs Ojide CK and Nwadike VU were involved in
207 literature search, statistical analysis and drafting of the manuscript. All the authors read and approved
208 the final manuscript.

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