High seropositivity of IgG and IgM antibodies against cytomegalovirus (CMV) among HIV-1 seropositive patients in Ilorin, Nigeria

Adeola Fowotade1, Iheanyi Omezuruike Okonko2, Olajide Olubunmi Agbede3, S T Suleiman3

1. Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Nigeria
2. Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Choba, East-West Road, Port Harcourt, Nigeria 500102
3. Department of Medical Microbiology & Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria

Abstract

Background: Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) is a major public health problem in sub-saharan Africa. Cytomegalovirus (CMV) has been reported to enhance HIV replication and accelerate the progression of HIV infection to AIDS.

Objective: This study reports on the high seropositivity of immunoglobulin (Ig) G and M antibodies against CMV and the risk factors for CMV infection among HIV/AIDS patients in Ilorin, Nigeria.

Method: A total of 180 consented HIV-1 seropositive patients (age-range 16-56 years; 108 females and 72 males) were consecutively recruited. Socio-demographic/behavioral data and 5 ml blood samples were collected from each patient. Plasma of each sample was assayed for anti-CMV IgG/IgM using a CMV IgG and IgM Enzyme Linked ImmunoSorbent Assay (ELISA) kit.

Results: Twenty (11.1%) of the 180 HIV-1 seropositive subjects were positive for anti-CMV IgM antibody while 169(93.9%) were positive for anti-CMV IgG antibody. Age, marital status, number of sexual partners, CD4 cells counts and previous history of blood transfusion were the main correlates of CMV seropositivity among these patients. However, occupation, sex, highly active antiretroviral therapy (HAART) were not statistically associated with CMV seropositivity in this study.

Conclusion: This study has shown that greater percentages of HIV-1 seropositive patients had active CMV infection. It has further shown that CMV is hyperendemic in HIV-1 seropositive patients in Ilorin, Nigeria.

Keywords: CD4, CMV, HIV/AIDS, IgG, IgM, Risk factors, HAART

DOI: http://dx.doi.org/10.4314/ahs.v15i1.1

Introduction

Human cytomegalovirus (HCMV) is a ubiquitous agent that can cause infection at any time during the course of life and commonly infects individuals from diverse geographical and socio-economic backgrounds1-2. By serology, 30% to 100% of the general population exhibit prior exposure to the virus3. The virus often causes asymptomatic infection in healthy persons; when symptomatic, HCMV infection presents with three recognizable clinical syndromes4.

HCMV is also a virus most frequently transmitted to developing foetus, causing birth defects in new born and immune defect in later life and increase morbidity and mortality5. About 2.0% of pregnant women have either a primary or a restricted HCMV infection during pregnancy and it is estimated that 10-20% of congenitally infected newborns show evidence of the infection6.

Infections by HCMV continue to be an important health problem in certain patient populations, such as newborns, recipients of solid organs or bone marrow and AIDS patients. In these groups, HCMV is a major cause of morbidity and mortality. In various parts of the world, the prevalence of HCMV ranges from 40-100%2. The risk of exposure to HCMV increases with age3. As with other herpes viruses, HCMV remains latent in the infected host throughout life and rarely reactivates to cause clinical illness except in immunocompromised individuals7-9.

HCMV infection is more prevalent in populations at
Prior to the introduction of combination antiretroviral therapy (cART), HIV infection was commonly transmitted through blood transfusion. Transfusion of blood from infected donors can transmit HIV virus to the recipient. Human Immunodeficiency Virus (HIV) incidence among people living with HIV/AIDS in Ilorin and its environment is noted in Table 1.

**Methods**

**Study area**

This prospective study was carried out at the University of Ilorin Teaching Hospital (UTH), Ilorin, Northern Nigeria. The teaching hospital provides healthcare services to the people of Kwara and neighboring states. UTH is in conjunction with the Institute of Human Virology (IHN) and provides free health care services to people living with HIV/AIDS in Ilorin and its environment.

**Ethical consideration**

A written consent was obtained from participants after careful explanation of the concept of the study to them. Ethical clearance was sought and obtained from the ethical and research committee of the University of Ilorin Teaching Hospital, Ilorin, Nigeria.

**Experimental design**

A total of 180 consented HIV seropositive patients attending the HAART clinic of UTH, Ilorin, were recruited for this study. The demographic data of the participants were entered into a structured question-naire designed for the study. A serological survey was done by collecting blood samples from all patients for HCMV IgG and IgM. These samples were sent to Giostyle box with ice packs to preserve the cold chain to the laboratory. Serum was extracted from each sample by centrifugation at 3000 rpm for 5 minutes using ESS centrifuge. All the sera obtained were stored frozen at -20°C until analysis was done.

**Blood collection and serological analysis**

Five milliliters of blood was collected into sterile anticoagulant-free bottle. Each sample was centrifuged after the blood had clotted and serum separated into sterile bottles on each collection day for storage at -20°C. All specimens were screened for HCMV specific-IgM and IgG antibodies using IgM and IgG ELISA Kit manufactured by DIA PRO Diagnostic Bioprobes Srl Via Columella nO31 20128 Milano – Italy. The tests were done according to manufacturer’s instructions. The cut-off of the device was set at 0.5 WHO IU/ml (Calibrator 2) by the kit’s manufacturer. Samples with a concentration higher than 0.5 WHO IU/ml were considered positive for CMV IgG whilst samples with concentration below the cut-off were regarded as negative results.

**Sensitivity and specificity of the Elisa kits**

The value, obtained from the analysis of more than 300 specimens, has been > 98% of sensitivity. An overall value > 98% of specificity was found when examined on more than 100 specimens.

**Data analysis**

Data was analyzed using Microsoft Excel 2007 version to calculate the International Unit (IU) from Optical Density (OD). Values below 0.5 were considered negative and values above 0.5 were considered positive. Statistical Package for Social Sciences (SPSS, version 19.0) software was used to calculate descriptive statistics.

**Results**

**Patients’ characteristics**

Of a total of 180 consented HIV-1 seropositive patients that participated in the study, 108 (60.0%) were females while males were 72 (40.0%) in number giving a male to female ratio of 1:1.3. Socio-demographic data of HIV-1 seropositive patients tested for anti-CMV IgG/IgM antibodies are shown in Table 1 and 2. Behavioral data of HIV-1 patients tested for anti-CMV IgG/IgM anti-bodies are shown in Table 3 and 4.

**Distribution of anti-HCMV IgM antibody among HIV-1 seropositive subjects**

Twenty (11.1%) of the 180 HIV-1 seropositive subjects were positive for anti-CMV IgM antibody while 169 (88.9%) were positive for anti-CMV IgG antibody (Table 1). The value obtained from the analysis of more than 300 specimens was 0.5 IU/ml. The cutoff of the device was set at 0.5 WHO IU/ml (Calibrator 2) by the kit’s manufacturer. Samples with a concentration higher than 0.5 WHO IU/ml were considered positive for CMV IgM whilst samples with concentration below the cut-off were regarded as negative results.

**Table 1: Socio-demographic data and seropositive outcome of HIV-1 patients tested for anti-CMV IgG/IgM antibodies**

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. Tested (%)</th>
<th>Positive (%)</th>
<th>Statistical Values</th>
<th>Statistical Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-25</td>
<td>7(3.9)</td>
<td>1(14.3)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>26-35</td>
<td>74(41.1)</td>
<td>10(13.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-45</td>
<td>81(45.0)</td>
<td>8(9.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 and above</td>
<td>18(10.0)</td>
<td>1(5.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>108(60.0)</td>
<td>90(92.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>72(40.0)</td>
<td>10(13.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>39(21.7)</td>
<td>5(12.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>127(70.5)</td>
<td>112(98.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>147(80.8)</td>
<td>13(92.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civil servants</td>
<td>20(11.1)</td>
<td>1(5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traders</td>
<td>100(55.6)</td>
<td>100(100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>10(5.5)</td>
<td>10(100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>30(16.7)</td>
<td>27(90.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>20(11.1)</td>
<td>1(5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>180(100.0)</td>
<td>169(93.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Anti-CMV IgG/IgM antibody positivity outcome of HIV-1 seropositive subjects**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Anti-CMV IgG</th>
<th>Positive (%)</th>
<th>Statistical Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-25</td>
<td>1(14.3)</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>26-35</td>
<td>10(13.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-45</td>
<td>8(9.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 and above</td>
<td>1(5.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Distribution of anti-HCMV IgM antibody among HIV-1 seropositive subjects**

Twenty (11.1%) of the 180 HIV-1 seropositive subjects were positive for anti-CMV IgM antibody while 169 (88.9%) were positive for anti-CMV IgG antibody (Table 1). Also from Table 1, it can be observed that there was no significant difference (X2 = 1.434, p-value = 0.591) in the seropositivity outcome of anti-CMV IgG antibodies among the subjects (Table 1). The level of anti-CMV IgM and IgG antibodies seropositivity among the various marital groups was also statistically significant (X2 = 3.906, p-value = 0.002) (Table 1). The prevalence of anti-CMV IgM was higher in singles (97.4%) than their married counterparts (92.9%) and others (92.7%) as shown in Table 1.

**Table 3: Distribution of anti-CMV IgG/IgM antibody positivity outcome of HIV-1 seropositive subjects**

There was no significant difference (X2= 1.784 p-value=0.629) in the seropositivity outcome of anti-CMV IgM antibody among the various sex groups. The prevalence of anti-CMV IgM was higher in males (13.9%) than their female counterparts (9.3%). In the same vein, the prevalence of anti-CMV IgG was also higher in males (95.8%) than females (92.6%) as shown in Table 1.
was higher among traders (16.0%), followed by farmers (10.0%), civil servants (5.0%) and unemployed subjects (5.0%) while other occupations had the least prevalence of anti-CMV IgM (3.3%). In the same vein, the prevalence of anti-CMV IgG was higher among traders (100.0%) and farmers (100.0%), followed by other occupations (90.0%), civil servants (80.0%) and unemployed subjects (80.0%) as shown in Table 1.

**Behavioral data and seropositivity outcomes of HIV-1 seropositive patients tested for anti-CMV IgG/IgM antibodies**

Table 2 shows the behavioral data and seropositivity outcomes of HIV-1 seropositive patients tested for anti-CMV IgG/IgM antibodies. Our study also found significant difference (X²=24.25, p-value=0.000) in the seropositivity of anti-CMV IgM and IgG antibodies among those with single (8.5%) for IgM and 86.4% for IgG) and multiple sexual partners (12.4% for IgM and 97.5% for IgG) as shown in Table 2. The study shows no significant difference (X²=0.80, p-value=0.777) in the level of anti-CMV IgM and anti-CMV IgG antibodies among the HIV-1 seropositive patients on HAART (Table 2). The prevalence of anti-CMV IgM was higher among subjects who were not on HAART (54.4%) than those on HAART (45.0%). In the same vein, the prevalence of anti-CMV IgG was higher among subjects who were not on HAART (94.4%) than those on HAART (90.0%) as shown in Table 2. The results showed that of the 40 (22.2%) subjects with previous history of blood transfusion; 6(15.0%) were seropositive for anti-CMV IgM and 38(90.0%) for anti-CMV IgG antibody. While among those with no history, 14(10.0%) were positive for anti-CMV IgM and 131(93.6%) for anti-CMV IgG antibody. There was significant difference (X²=1.412, p-value=0.000) in the level of anti-CMV IgM and anti-CMV IgG antibodies among the two groups (Table 2). The CD4 cell counts ranged from 17 - 321 cells/mm³. There was significant association (X²=1.155, p-value=0.0000) between CD4 cells count and seropositivity outcome of HIV-1 seropositive patients tested for anti-CMV IgM and anti-CMV IgG antibodies (Table 2).

**Discussion**

Cytomegalovirus (CMV) is a very frequent infection complicating AIDS. Sexual transmission appears to be the most common route of infection in adults, though CMV can also be spread through ophthalmological sections, urine, breast milk, and blood.

CMV-specific antibody of the IgM class is a marker of active or recent primary infection with the virus. Post-transfusion CMV infection correlates positively with the receipt of blood from CMV IgM-positive donors. A decreased incidence of Transfusion-associated CMV infection was reported when only blood products negative for CMV IgM were used. Most patients with AIDS who develop clinical signs and symptoms of CMV infection probably have reactivation of previous infection rather than primary infection. The prevalence of HIV/AIDS in Sub-Saharan Africa is high but the description of CMV infection as opportunistic infection amongst patients is scanty.

The aims of this study were to determine the prevalence of CMV infection in HIV positive patients with and without past history of blood transfusion and compare our findings with those of other studies. The study reports that 11.1% of the HIV-1 seropositive patients tested were anti-CMV IgM seropositive and 93.9% were positive for anti-CMV IgG antibody.

This finding is in consonance with what has been previously reported. Akinsola et al. reported few cases of CMV reinitis in HIV infected Nigerians. The HIV patients who developed symptomatic CMV infection may have had the infection for a long time; immunosuppression by HIV makes the virus to become pathogenic. The higher the prevalence of CMV in the general population, the higher should be the prevalence of CMV infection in the population of HIV infected patients.

The 93.9% reported for anti-CMV IgG antibody seropositivity in this study is comparable to the values reported in previous studies in Nigeria and outside. The seroprevalence of CMV IgG of 96.0% was reported by Akinshai et al. in Lagos, Nigeria. Okwori et al. reported a seroprevalence of 84.2% among pregnant women. Similar seroprevalence rates of 90.0 – 100.0% were also found in India. The high seroprevalence in Nigeria and India contrasts with Western literature, in which seroprevalence ranges from 38.0% to 75.0%. A seroprevalence of 40.0% was found in highly industrialized nations. Uyar et al. reported a 97.3% and 1.0% seropositivity for anti-CMV IgG and anti-CMV IgM antibodies respectively in Northern Turkey.

The seroprevalence of CMV IgG of 100.0% among healthy blood donors was also found in the study of Kreeb done at Ibadan, Nigeria in 1973. A high seroprevalence of between 90 -100% was also found in India amongst immunocompetent subjects in various studies. Atul and Ramanchandrum found 95.0% seroprevalence of CMV IgG amongst blood donors. A study by Pal et al. in 1972 showed 100.0% seropositivit for CMV IgG in a population of immunocompetent adults. Madhavan et al. in 1974 showed that 84-96% of immunocompetent adults had the antibody.

The study showed that the seropositivity of anti-CMV IgG and anti-CMV IgM antibodies were age dependent. The positivity for anti-CMV IgG and anti-CMV IgM antibodies was not found to be the same in all age groups. The age-related distribution of anti-CMV–specific IgM antibodies among the HIV-1 seropositive subjects showed a significant difference in the levels of anti-CMV IgG and anti-CMV IgM antibodies among the various age groups tested (p=0.000). This is also in keeping with the findings of previous studies. CMV infections occurs worldwide, about four out of five people over age 35 have been infected with cytomegalovirus, usually during childhood or adulthood. In most of these people, the disease is so mild that it is overlooked.
Though, there was no significant difference (p=0.591) in the level of anti-CMV IgG and IgM antibodies among the subjects, sex-related distribution of CMV IgM among the HIV-1 seropositive subjects showed that of the 108 females tested, 10(13.9%) were anti-CMV IgM positive and 98(88.1%) were anti-CMV IgM negative. Also from Table 1, it can be observed that of the 108 females tested, 100 (92.6%) were anti-CMV IgG positive and 6 (5.8%) males were positive for anti-CMV IgG. Existing evidence suggests that the concentrations of IgG immunoglobulin in maternal and cord sera are essentially the same.\(^{27}\)

In this study, a seroprevalence of 92.6% for CMV IgG and 9.3% for CMV IgM was observed in females. This is a deviation from what was reported by earlier workers in Nigeria and outside Nigeria\(^{17,27-30}\). In Brazil, 94.7% prevalence rate was reported among females\(^{31}\). In Gambia, 14.0% of 178 Gambian babies were congenitally infected despite the fact that 87.0% of their mothers were antibody positive to CMV\(^{32}\). At the time of delivery of 96.0% of the 150 Egyptian mothers and their newborn infants were CMV-IgG seropositive\(^{33}\). The implication of these findings is that individuals with CMV antibodies and their newborns are at high risk of developing the virus if not protected. The detection of virus-specific IgG and IgM antibodies in relation to marital status showed statistical association (X²= 1.306, p-value=0.002) in the anti-CMV seropositivity outcome of anti-CMV IgM and anti-CMV IgG antibody. In this study, anti-CMV IgG positive and 69 (95.8%) were anti-CMV IgG seropositive and 118 (97.5%) were anti-CMV IgG antibody positive. There was no significant difference (X²= 0.080, p-value=0.777) in the level of anti-CMV IgG antibody positive and 69 (95.8%) were anti-CMV IgG positive, compared with the 12.8% seropositivity observed among singles and 7.1% among others (widows/widowers/divorced). Also, 118 (92.9%) of the married were anti-CMV IgG positive and compared to 97.4% seropositivity among singles and 92.7% among others.

In term of their HAART status, it can be seen that 45.0% of the 20 that were CMV IgM positive were already on HAART, while 87 (54.4%) out of the 160 that were not yet on HAART were also found to be CMV IgM seropositive. There was no significant difference (X²= 0.080, p-value=0.777) in the level of CMV IgM among the HIV-1 seropositive individuals that were on HAART and those that were not. CMV-specific antibody of the IgM class is a marker of active or recent primary infection with the virus. Transmission of CMV is also congenital (in birth), through maternal placenta\(^{25,31}\). CMV infection constitute a real issue for the developing fetus, if pregnant. This may be due to the fact that IgG antibody is unique among the major immunoglobulin classes for its active transfer across maternal placenta\(^{33,31}\).

Transmission of CMV is sexual\(^{34}\). Among the 59 (32.8%) HIV-1 seropositive individuals with single sexual partners, 5(8.5%) were anti-CMV IgM seropositive and 51 (86.4%) were anti-CMV IgG seropositive. Of the 121 (67.2%) HIV-1 seropositive individuals with multiple sexual partners, 15 (12.4%) were CMV IgM seropositive and 118 (97.5%) were anti-CMV IgG seropositive. The study statistical association in the seropositivity outcome of anti-CMV IgM and anti-CMV IgG antibodies and the number of sexual partners. This study also determines the immune status of HIV-1 seropositive patients to CMV in Ilorin. In the studied population, 88.9% of the HIV seropositive patients and 97.4% of the HIV negative controls were immune to CMV. The implication of these findings is that individuals seronegative for CMV are susceptible to CMV primary infections\(^{35}\). The CD4 cell counts-related distribution of CMV IgM and IgG antibodies among the re-

<table>
<thead>
<tr>
<th>References</th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| 8. Krech U. Complement-fixing antibodies against cyto-
| 9. Greffe A, van der Giessen M, van Son W, The TH. Circulating cytomegalovirus (CMV)-infected en-
| 10. | 11. | | 12. |

# Conclusion

This study has shown that greater percentages of HIV-1 seropositive patients had active CMV infection. It has further shown that CMV is hyperendemic in HIV-1 seropositive patients in Ilorin, Nigeria. Unfortunately, vaccines for CMV have not yet been developed\(^{37}\). Preventive measures must be taken to decrease the morbidity and mortality related to CMV infections\(^{37}\). There is therefore need to routinely screen blood donors and pregnant women for evidence of CMV infection during their transfusion and antenatal visits respectively.

# References

9. Greffe A, van der Giessen M, van Son W, The TH. Circulating cytomegalovirus (CMV)-infected en-


22. Bernard JT. Human Cytomegalovirus Species. Classification of Viral Family (Herpesviridae), 2007; 2127-128


