

Original Research Article

SEROPREVALENCE OF CYTOMEGALO VIRUS INFECTION AMONG HIV PATIENTS ACCESSING HEALTHCARE IN FEDERAL MEDICAL CENTRE KEFFI, NIGERIA

ABSTRACT

Background Cytomegalovirus (CMV) a herpes virus known for latency after primary infection is a major cause of morbidity and mortality in HIV/AIDS patients. It is reported to enhance HIV replication and acceleration of HIV infection to AIDS. **Aim** There is a dearth of published information on the prevalence of CMV infection among HIV/AIDS patients in this area. This cross sectional study was designed to determine the prevalence and risk factors for the viral infection among HIV/AIDS patients in Keffi. **Method** Blood samples from 208 HIV patients were screened for CMV using specific CMV IgG Enzyme Linked Immunosorbent Assay (Cortez diagnostic Inc, USA) according to the manufacturer's instructions.. **Results** The overall prevalence of CMV IgG antibody was 77.0%. The prevalence of viral infection based on sex was found to be 82.6% among the males and 75.3% in females ($P > .05$). Seroprevalence was found to be highest among those aged 10 – 20 years (90.9%) and least among those aged 41-50 years (68.8%) ($P > .05$) There was a statistically significant association between the viral infection and CD4 cells count ($P < .05$). HIV patients with CD4 of < 100 cells/ reported the highest prevalence (100%). There was a decrease in prevalence with an increase in CD4 cell counts. **Conclusion** This study reported 77.0% CMV infection among HIV patients with low CD4 counts as a risk factor. Marital status, occupation, level of education, residence and antiretroviral therapy status had no statistically significant association with CMV infection ($P > .05$). The threat of CMV reactivation and consequent sequelae among those seropositive to IgG must not be overlooked in the study population. HIV patients should therefore be monitored closely for clinical signs of CMV syndrome.

Key words: Cytomegalovirus, HIV, CD4, seroprevalence, Keffi

INTRODUCTION

Cytomegalovirus (CMV) was first described when inclusion bearing cells were shown by Ribbert while Goodpasteur and Talbert were the first to suggest that this cytomegalia (inclusion cells), could be due to a viral agent. Subsequently, Rowe et al. and Weller et al. independently isolated the human CMV strains and in 1960, Weller et al. proposed the term “cytomegalovirus” and then isolated HCMV from the urine of infants with generalized disease [1]. It is a member of a family of 8 human herpes viruses (HHV) designated as HHV type 5 The virus attacks the cells

of the immune system, particularly the CD4+ T lymphocytes resulting in their depletion. Although, infection with CMV is self-limiting in immunocompetent individuals, it is associated with high morbidity and mortality in those immunocompromised [1]

CMV is a ubiquitous virus and its infection occurs mainly in childhood in most populations [2] especially in sub Saharan Africa [3]. It is a slow replicating virus, infecting only as many as 1% of all neonates in developed countries, but demonstrating up to 90% -100% IgG-positivity in developing countries like Africa and Asia [4, 5]. Primary infection is defined as CMV infection in a previously seronegative person whereas secondary infection is defined as intermittent excretion of the virus in the presence of host immunity and may be due to either reactivation of an endogenous virus or exposure to a new virus strain from an exogenous source [6]. It is a widely distributed opportunistic agent seen with AIDS [4, 7].

In most people with a fully functional immune system, the initial infection with CMV may cause a mild flu like illness and later the virus remains dormant. A damaged immune system permits the reactivation of CMV. A synergistic effect may worsen the progression in HIV infected persons especially as CMV is known to replicate more rapidly in the presence of HIV [3]. The Human Immunodeficiency Virus (HIV) has remained a major public health problem all over the world. In fact the United States program on HIV/AIDS (UNAIDS) estimated that there were over 35.3 million people living with HIV by the end of 2012 [8]. It has also been noted that majority of the diseases caused by CMV are related to the reactivation of the latent infection [7]. Human Cytomegalovirus (CMV) infection can be life- threatening for the immunocompromised, such as HIV-infected persons . This necessitated the present study among HIV patients in Keffi, Nigeria.

MATERIALS AND METHODS

Study Area and Population

The area of study for this research work was Keffi. It is approximately 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the Capital of Nasarawa State. Keffi town is located between Latitude 8⁰5'N of the Equator and Longitude 7⁰8'E and situated on an altitude of 850m above sea level [9].

The study population was adults living with HIV and accessing healthcare in Federal Medical Centre, Keffi who agreed to participate in the study. Some were on HAART while others HAART naïve. They were of both sexes and aged 18-60 years. On the whole, 208 people consented to take part in the study. The socio-demographic information of the participants was obtained by use of oral interview.

Type of study

This was a cross sectional study that was carried out from January to April 2014.

Sample Collection

About 5ml of blood was collected from each participant by venepuncture and dispensed into a plain universal container. It was allowed to clot for 30 minutes, centrifuged at 3000rpm for 5 minutes and the sera were transferred into respective labeled plain containers by the aid of a sterile Pasteur pipette. All the samples were then transported to the Innovative Biotech Limited Keffi for screening .

Laboratory Investigation

Specific Cytomegalovirus IgG (CMV IgG) ELISA test kit (Cortez Diagnostics Inc. USA) was used according to the manufacturer's instructions.

Principle of the Test

Purified CMV antigen is coated on the surface of microwells. Diluted patient serum was added to the wells and the CMV IgG specific antibody, if present binds to the antigen. All unbound materials are washed away. Excess enzyme conjugate was washed off and Tetramethylbenzidine (TMB) chromogenic substrate was added. The enzyme conjugate catalytic reaction was stoped after a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader and compared in a parallel manner with calibrator and controls.

Assay Procedure

The desired number of CMV-antigen coated strips of microtitre wells were placed into the holder and 1:40 dilution of each negative control, positive control and calibrator was prepared by adding 200µl of sample diluents to 5µl of each of the reagents and mixed properly. 100µl of the

diluted sera, calibrator and controls were dispensed into appropriate wells. For the reagent blank, 100µl sample diluents were dispensed into the well in position A1. The holder was tapped gently to remove air bubbles from the liquid and also to mix the contents of each well. The test strips were incubated for 30minutes at room temperature. After incubation, the liquid content was removed and further dapped onto tissue paper pad. During each wash, 100µl of washing buffer was dispensed into the test wells and poured off. After the third wash, 100µl of TMB chromogenic substrate was dispensed into each well and incubated again for 30minutes at room temperature. After which 100µl of stop solution was added to stop the reaction. A microwell reader was used to read the optical density at 450nm (BIORAD).

Interpretation of the Result

This was carried out according to the manufacturer's recommendations.

Negative Result: When CMV G index of 0.90 or less, IgG antibody to CMV is considered to be negative.

Equivocal Result: CMV G index of 0.90-0.99 is said to be equivocal, and the sample should be retested.

Positive Result: CMV G index of 1.00 or greater, or IU value greater than 1.2 are obtained, the result is said to be positive for CMG IgG antibody.

Statistical Analysis

Data obtained from the study were analyzed using Chi-Square (χ^2) using statistical software (SPSS Inc, Version 17, Chicago, USA) to determine the association between prevalence of infection and the studied parameters. Values obtained were considered statistically significant at $P \leq .05$.

RESULTS

A total of 208 HIV infected participants were recruited for this study made up of males and females aged 10 – 60 years old. Of these, 160 (77.0%) were reactive to anti- CMV IgG. As shown in Table 1 the highest prevalence of infection for the different parameters studied was

among those aged 10 -21 years, farmers, and illiterates ($P > .05$). With respect to gender, marital status and Anti retroviral therapy status, viral infection was higher among males, unmarried participants and those that were not on antiretroviral therapy respectively ($P > .05$). The only parameter that was significantly associated with infection prevalence was CD4 counts. Prevalence was highest among those with counts of less than 100 cells/ μ l .

Table 1: The Seroprevalence of CMV infection in respect to risk factors among HIV patients accessing healthcare at FMC Keffi.

Variables	No. Screened	No. Infected (%)	<i>P</i> -value
Age (yrs)			
10-20	11	10(90.9)	
21-30	118	88(74.6)	
31-40	56	45(80.4)	
41-50	16	11(68.8)	$P > .05$
51-60	7	6(85.7)	
Sex			
Male	46	38(82.6)	$P > .05$
Female	162	122(75.3)	
Marital Status			
Single	75	59(78.7)	$P > .05$
Married	133	101(75.9)	
Residence			
Urban	126	95(75.3)	$P > .05$
Rural	82	65(79.3)	
Occupation			
Farmers	16	14(87.5)	$P > .05$
Housewives	33	26(78.8)	
C/Servants	19	12(63.2)	
Students	56	44(78.6)	
Artisans	84	64(76.2)	
ART Status			
HAART	153	116(75.8)	$P > .05$
HAART naïve	55	44(80.0)	
Educational Level			
Illiterate	29	24(82.8)	
Primary	113	91(80.5)	$P > .05$
Secondary	44	30(68.2)	
Tertiary	22	15(68.2)	
CD4⁺ (Cells/μl)			
≤ 100	9	9(100)	
101-200	22	17(77.3)	
201-300	31	25(80.6)	

301-400	52	42(80.8)	<i>P</i> < .05
401-500	48	40(83.3)	
501-600	24	15(62.5)	
601-700	11	7(63.6)	
≥ 701	11	5(45.4)	

123

124 **DISCUSSION**

125 This study determined the seroprevalence of CMV infection among HIV patients using anti- IgG
126 antibodies to CMV as the seromarker. Seroprevalence of anti-CMV IgG was 77.0% among the
127 HIV-infected patients in the present study. This is lower than prevalence rates in similar studies
128 in Nigeria where researchers reported 100% in Lagos [4], 100% in Maiduguri [5], 97.0% in
129 Kano [10], 98.8% in Benin [11], and 93.9% in Ilorin [12]. However lower prevalence rates
130 have also been reported in other Nigerian studies as 13.0% in Kano [13], 75% [14] and 14.8%
131 [15] in Lagos. In similar studies, researchers from other countries have reported 10.4% from
132 India [16], 97% from Indonesia [7] and 95.6% among HIV positive Malawian children [3].
133 These differences in prevalence might not be unrelated to geographic, ethnic and social factors as
134 posited by Sufiawati et al. [7]. Other reasons might be the characteristic of the study population
135 and the sensitivity of the screening tests.

136 This study also revealed that male subjects living with HIV had a higher prevalence of CMV
137 infection than their female counterparts; males subjects had a prevalence rate of 82.6% and the
138 females 75.3%. There was no statistically significant association between the seropositivity of
139 CMV-IgG and gender (*P* > .05). This outcome is in consonance with the report of Fowotade et
140 al. [12] where they recorded a higher seroprevalence of the viral infection among male than the
141 female subjects but is in contrast with the outcome of the studies of Musa et al. [13] and Ojide et
142 al. [11] who found that the females had a higher prevalence rate than their male counterparts.
143 Similar to the present study none of these studies reported a significant association between viral
144 infection and gender.

145 Seropositivity of CMV infection in relation to age revealed that prevalence was highest among
146 those aged 10 - 20 years (90.9%) and least among those aged 41-50 years (68.8%).(*P* > .05) On
147 the whole, there was a high seroprevalence in all the age groups. The possibility that most of

these patients were infected even before they became immunocompromised cannot be ruled out especially as CMV is known to be ubiquitous and said to be a common childhood infection [2, 3].. This might also signify the endemicity of CMV in the study area. Similarly, Fowotade et al. [12] and Olajumoke et al. [14] did not find any significant relationship between age and infection prevalence.

When stratified by marital status, the viral infection was not associated with being married or single. There was an IgG seroprevalence of 78.7% among the unmarried and 75.9% among the married which did not show any significant difference ($P > .05$). This might not be entirely unrelated with the fact that non-sexual transmission has been reported as a primary mode of transmission in Nigeria [5]. Similarly, there was no statistically significant association between residence and the viral infection ($P > 0.05$) although seroprevalence of CMV infection was higher among rural (79.3%) than the urban participants (75.3%). Akinbami et al.[4] and Kida et al [5] noted that seroprevalence of CMV IgG can be as high as 90 - 100% in African populations. This high endemicity of the virus could have caused the indifference in prevalence with respect to residence.

With reference to occupation, farmers recorded the highest seroprevalence of infection (87.5%), followed by housewives (78.8%), students (78.6%), artisans (76.2%) and the least seroprevalence was recorded among civil servants (63.2%). There was no statistically significant association between the viral infection and occupation. Others have noted that there were more CMV seropositives among the traders and farmers and lower in civil servants and unemployed subjects [12]. In a related development, this study reported viral infection was highest among those with the lowest level of education and lowest among those with secondary and tertiary education ($P > .05$) but with consistently high prevalence irrespective of educational level attained. However, others have reported lack of education as one of the factors responsible for CMV acquisition [5].

The distribution of viral infection in relation to anti retroviral status revealed that highly active anti retroviral therapy (HAART) naïve patients had a higher prevalence rate (80.0%) compared to those on HAART (75.8%). However, there was no statistically significant association between antiretroviral treatment status and the viral infection ($P > .05$). But there was a statistically

significant association between CD4 cell counts and the prevalence of CMV infection among the study participants ($P < .05$). The seroprevalence of the viral infection was highest among those participants with CD4 cells of less than 100 cells/ μ l (100.0%) while the lowest was recorded among participants with ≥ 701 (45.4%) CD4 cell counts. Although infection prevalence was generally high irrespective of CD4 counts having very low CD4 counts was a predisposing factor to infection. This report is in consonance with the work of Musa et al. [13] which recorded highest susceptibility among participants with < 100 CD4 cells/ μ l) and least susceptibility was shown in > 356 cells count. However, being immunocompromised portends the possibility of viral reactivation with its attendant consequences.

CONCLUSION

A relatively high prevalence (77.0%) of Cytomegalovirus infection was reported in the present study. The high prevalence suggests endemicity of this viral infection in the study area. This is a cause for alarm especially as the threat of CMV reactivation with its attendant clinical syndromes cannot be overlooked. Only CD4 count was found to be statistically associated with viral infection prevalence, other risk factors remained elusive. Public health education programs on general hygiene for HIV-infected people should be put in place while CMV IgG seropositives should be closely monitored for any signs of CMV syndromes.

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