Prevalence of cytomegalovirus among HIV patients attending Sobi Specialist Hospital, Ilorin, Nigeria

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Abstract

Human immunodeficiency -virus (HIV) is an endemic pathogen in several middle and low-income countries. The pathogen creates a state of host immunosuppression and immunocompromised that predisposes an individual to reactivation of latent pathogens such as herpesviruses. Cytomegalovirus (CMV) are opportunistic viruses that invade an immune system which has been compromised by HIV, leading to co-infection. Hence, this study was carried out to determine the seroprevalence of cytomegalovirus among HIV-positive patients attending Sobi Specialist Hospital, Ilorin, Nigeria. A total of 200 HIV-positive patients were recruited into the study. Confirmation of HIV status of recruited patients was done using rapid diagnostic test kit and patients' CD_4^+ cell estimation was carried out using an automated cyflow CD_4^+ cell counter. Analysis of CMV-specific IgM was done using enzyme-linked immunosorbent assay (ELISA). Of the 200 HIV-positive patients, 58 (29%) were male while 142 (71%) were female. HIV was most prevalent among individuals 31–40 years old (35%) and married individuals (76%). The prevalence of risk factors associated with HIV infection included heterosexuality (33.5%), blood transfusion (31.5%), injection drug users (25%), congenital transmission (7.5%), and organ transplant (2.5%). The CD_4^+ cell count of HIV-positive patients was > 350 (56.5%), 100–350 (33.5%), and < 100 (10%). Only 3 HIV-patients (1.5%) were seropositive for CMV. Overall, the study confirmed that the prevalence of CMV among HIV-infected patients is generally low.

Keywords: CD_4^+ Cells, ELISA, IgM antibodies, Seroprevalence, Co-infection

1. Introduction

Human cytomegalovirus (HCMV) is a significant member of the Herpesviridae, belonging to the sub-family β-Herpesvirinae. The mature HCMV virion has a diameter of 150–200 nm, icosahedral symmetry, and a double-stranded DNA genome of 230 kbp. The HCMV envelope contains a cellular lipid bilayer composed of viral glycoproteins [1,2]. CMV can remain viable for several hours on different environmental surfaces including metal and wood (1 hour), glass and plastic (3 hours) and cloth (6 hours). It can be kept at 4°C for a few days without losing its infectivity and can be stored for several months at -70°C [3]. CMV can be transmitted both horizontally or vertically with varying pathological impact such as systemic inflammation, hepatitis and immune dysregulation in the host [4]. CMV is one of the most successful human pathogens that has little effect on the host with transmission occurring in utero, perinatally or postnatally. The routes mostly speculated to aid transmission are saliva and sex [4]. However, saliva is the main route of CMV transmission postnatally which accounts for the high prevalence amongst children [4]. Recurrent maternal infection or primary infection can result in vertical transmission of HCMV to the foetus [5]. Infected genital secretions and breast milk are two significant routes by which perinatal CMV infection is contracted [6]. CMV reinfection can also cause post-primary infection when an individual contracts another or the same strain of CMV [7]. Globally, CMV has a 2–10% prevalence among infants under 6 months [8]. Though the precise mechanism of transplacental passage is unknown [9].

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The human immunodeficiency virus (HIV) is a pathogen of global significance that is commonly transmitted via unprotected sex, infected blood and bodily fluids contaminated with the blood of infected individuals. HIV infection in humans culminate in a diseased state called acquired immunodeficiency syndrome (AIDS). HIV is one of the factors that account for the high prevalence of AIDS infection in Sub-Saharan Africa [10]. *In vitro* and *in vivo* studies of HIV-1 infection have demonstrated that co-infection with herpesviruses can increase HIV replication through synergistic activation of the viral promoter regions in CMV and HIV [11]. Also, there are similarities in the pathogenesis of the two viruses and both pathogens are known to replicate within the epithelium of the genitourinary tract [12]. A positive correlation has been found between semen HIV-1 viral load and CMV DNA viral load. Higher viral load increases the risk of developing organ diseases which may result to death. High viral load is an indication that the virus has hijacked host immune system and is actively replicating [13]. Local immune activation is designed to provide protection against infection without introducing excessive amount of inflammation. CMV induces a pro-inflammatory state in the genital tract that activates HIV-1 replication, and anti-retrovirus therapy (ART) does not have an impact on this interaction [13]. Consequently, the links between CMV and HIV-1 are probably significant [13]. In CMV infected patients, there is an increased CMV-specific CD_8^+ T-cell responses once HIV infection has been established, even before the loss of peripheral CD_4^+ T cells [13]. CMV IgG may be associated with increased carotid artery stiffness and carotid artery lesions in HIV-infected women, with those on effective ART having a higher risk of developing vascular lesions in association with CMV co-infection [13]. The main aim of the present study is to ascertain the presence of specific CMV IgM antibodies in immunocompromised subjects bearing in mind that CMV is an opportunistic infection.

2. Materials and Methods

2.1 Ethical Consideration

The study was carried out at Sobi Specialist Hospital, a public healthcare centre in Ilorin, Nigeria. Approval for this study was obtained from the Ethical Committee of the hospital with a reference number KW/SH/ADM/61/V013/969C.

2.2 Study Population and Design

The study population included HIV-infected patients attending Sobi Specialist Hospital, Ilorin, Kwara State, Nigeria. Purposive cluster sampling method was adopted and a total of 200 outpatients with different demographic factors were employed for the study. The research lasted for six months from October 2015 to March 2016. Questionnaire were administered to obtain sociodemographic information and medical history of the patients.

2.3 Lentiviral Screening

The test was carried out using HIV test strip. Whole blood (2 ml) was collected from each patient by vein puncture into a vacutainer. Serum was separated after centrifugation of blood at 3000 rpm for 5 minutes. The serum was pipetted into the space provided on the strip and then left for **some minutes**. Appearance of two lines (control and test lines) indicates a positive result while appearance of a single line (control line) indicates a negative result [14].

2.4 CMV IgM Analysis

Whole blood (5 ml) was collected from each HIV-infected patient by vein puncture into an EDTA-containing vacutainer. The collected blood was separated into plasma and serum by centrifuging at 3000 rpm for 10 minutes and then stored in the refrigerator at -20 ºC. The separated serum was then analyzed for CMV-specific IgM using the ELISA. Purified CMV antigen was coated on the surface of microwells, and diluted patient serum was added to the wells for CMV-specific IgM. If present, it binds to the antigen while all unbound materials were washed away. Horseradish peroxidase-conjugate (HRP-conjugate) (100 µl) was added to the wells and bound to the antibody-antigen complex. Excess HRP-conjugate was washed off and 100 µl of tetramethyl benzidine (TMB) reagent was added. The enzyme conjugate catalytic reaction was stopped at exactly 10 minutes. The enzymatic reaction produced blue colour in positive control and CMV IgM positive sample well. The intensity of the colour generated was proportional to the number of IgM-specific antibodies present in the sample. The results were read by a microwell reader and compared in a parallel manner with the calibrator and controls. A CMV index ≤ 0.1 was considered negative and ≥ 0.1 was considered positive [14].

2.5 Estimation CD_4^+ Count

An automated Cyflow CD_4^+ cell counter was used to calculate the CD_4^+ count of study participants. CD_4^+ perierythene antibody (20 µl) and whole blood (20 µl) were gently mixed in a rohren tube and incubated in the dark for 15 minutes at room temperature. After incubation, 800 µl of CD_4^+ buffer was added to the tube with gentle swirling to mix. The sensor was then connected to the tube to begin counting.

2.6 Statistical Analysis

Statistical analysis was carried out using IBM SPSS (Version 21). Analysis for significant differences in the social demographic variables associated with the prevalence of HIV was tested using chi-square at a 95% confidence interval. All p-values less than 0.05 were accepted to be statistically significant.

3. Results

The age demographics of the recruited individuals are displayed in Table 1. The highest percentage of HIV-positive patients were between 31-40 years (35%), followed by 21-30 years (24%) and 41-50 years (22%). The least represented age group are individuals between 81 and 90 years (0.5%). Table 2 shows the prevalence of HIV in Sobi Specialist Hospital with respect to sex, marital status, and religin. Of the 200 HIV-positive individuals recruited into the study, 29% were male while 71% were female, 24% were single or unmarried and 76% were married, 23.5% were Christians and 76.5% were Muslims. According to the data, the prevalence of HIV was significantly higher in females, married individuals, and Muslims. Table 3 presents the risk factors associated with prevalence of HIV at Sobi Specialist Hospital. Heterosexuality (33.5%) was the most significant risk factor followed by blood transfusion (31.5%), injection drug users (25.0%), parent-to-child transmission (7.5%), and organ transplant (2.5%). The prevalence of HIV according to associated risk factors is statistically significant ($p<0.00001$).

Table 1: Age demographics of HIV-positive patients attending Sobi Specialist Hospital, Ilorin, Nigeria

Age (Years)	Frequency (n)	Percentage (%)
1-10	5	2.5
11-20	4	2.0
21-30	48	24.0
31-40	70	35.0
41-50	44	22.0
51-60	24	12.0
61-70	4	2.0
71-80	0	0.0
81-90	1	0.5

Table 2: Distribution of HIV patients according to sex, marital status, and religion at Sobi Specialist Hospital, Ilorin, Nigeria

Variable		Frequency	X^2	p-value
Sex	Male	58 (29.0)	35.28	< 0.00001
	Female	142 (71.0)		
Marital status	Single	48 (24.0)	54.08	< 0.00001
	Married	152 (76.0)		
Religion	Christianity	47 (23.5)	56.18	< 0.00001
	Islam	153 (76.5)		

Table 3: Prevalence of risk factors associated with HIV infection in HIV-positive patients at Sobi Specialist Hospital, Ilorin, Nigeria

Risk factors	Frequency	X^2	p-value
Blood transfusion	63 (31.5%)	51.646	< 0.00001
Organ transplant	5 (2.5%)		
Parent-to-child transmission	15 (7.5%)		
Heterosexual	67 (33.5%)		
Injection drug users (IDUs)	50 (25.0%)		

Table 4 shows the CD_4^+ cell count of HIV-positive individuals attending Sobi Specialist Hospital. Of the 200 HIV-positive patients included in the study, 56.5% had a CD_4^+ cell count greater than 350, 33.5% had CD_4^+ cell count between 100-350, and 10% had a CD_4^+ count less than 100. The prevalence of cytomegalovirus (CMV) among HIV-infected patients in this study area is presented in Table 5. Three patients representing 1.5% were seropositive for CMV while 197 (98.5%) were seronegative for CMV.

Table 4: CD_4^+ count of HIV-positive individuals attending Sobi Specialist Hospital, Ilorin, Nigeria

CD_4^+ count	Frequency	X^2	p-value
<100	20 (10.0%)	35.57	< .00001
100-350	67 (33.5%)		
>350	113 (56.5%)		

Table 5: Prevalence of CMV among HIV-positive patients attending Sobi Specialist Hospital, Ilorin, Nigeria

Age (Years)	CMV IgM positive	CMV IgM negative	Total
1-10	0	5	5
11-20	0	4	4
21-30	1	47	48
31-40	2	68	70
41-50	0	44	44
51-60	0	24	24
61-70	0	4	4
71-80	0	0	0
81-90	0	1	1
Total	**3 (1.5%)**	**197 (98.5%)**	**200 (100%)**

4. Discussion

HIV and CMV co-infection represent a significant public health concern. Being a herpesvirus, CMV undergoes latency in the bone marrow and can be reactivated by several factors, especially immunosuppression. Active HIV infection can grossly reduce the quantity of CD_4^+ cells in humans, thereby creating an immunosuppressed state in the host that favours reactivation of dormant pathogens, such as CMV. From this study, the prevalence of HIV in individuals between the ages of 21 to 50 is consistent with previous report [15]. This may be associated with several factors including unprotected sexual activities, multiple sexual partners, alcohol, and drug use, and underlying sexually transmitted infections. HIV prevalence was significantly higher among females (71%) than males (29%). In a similar study, Aguocha, *et al.* reported 29.9% and 70.1% HIV prevalence in males and females respectively in Imo State, Nigeria [16]. Higher HIV prevalence among females can be associated with the massive population of females compared to their male counterparts in the specific geographical region where the work was conducted. Also, higher seropositivity of HIV among married patients may be due to extramarital affairs among married couples while higher HIV prevalence among Muslims may be attributed to fact that Kwara State is largely dominated by Muslims.

Having multiple sexual partners (heterosexual) and blood transfusion were found to be significantly associated with HIV prevalence in this study with 33.5% and 31.5% respectively. Studies have shown sexual promiscuity and other sexually transmitted diseases to be associated with both HIV and CMV because of the shared route of transmission [17]. However, in a similar study, Ngangom *et al*. reported a lower HIV prevalence among heterosexual individuals, this variation can be due to a lack of proper equipment for screening blood samples and blood donors before blood collection [18].

The high CD_4^+ cell count recorded for more than half of the study population revealed that patients on high active anti-retroviral therapy (HARRT) who are regular with their check-ups and prescriptions have high CD_4^+ count which protect them from the risk of opportunistic infection by CMV. However, patients with low CD_4^+ count are either new to the clinic or not regular with their prescriptions. Hence, the HIV-CMV co-infection was observed to be dominant among this group due to the vulnerability of their immune system. A similar study reported CMV prevalent rates of 23%, 55.1% and 18.38% in patients with <100, 100-350 and >350 CD_4^+ cell count respectively [15]. The study also reported that CD_4^+ lymphocytes levels below 50 cells/µl are important markers in the prognosis of clinical manifestations of CMV and that they also indicate a disease phase which is frequently defined as advanced AIDS [15]. Variations in the CD_4^+ count can be associated with the viral load and immune status of the patient.

Akinsola *et al*. reported a few cases of CMV retinitis in HIV-infected Nigerians [19]. The HIV patients who developed symptomatic CMV infection may have had the infection for a long time; with immunosuppression by HIV causing the virus to become pathogenic. The higher the prevalence of CMV in the general population, the higher the prevalence of CMV infection among HIV-infected patients [20]. The presence of IgM antibodies may be due to primary infection, reactivation, or re-infection by CMV [21]. Similar studies in India also reported higher prevalence of 8% and 8.5% [22,23]. In another study, a CMV prevalence rate of 6.6% was reported among immunocompromised (HIV) patients attending Lagos University Teaching Hospital [20]. Variations in prevalence rates may be attributed to population size, geographical location, culture, and lack of proper preventive measures [24]. The high CMV antibodies observed in patients within the age bracket of 21-40 years could be attributed to the fact that this group represent active and sexually matured youths with the tendency towards sexual promiscuity and its resultant likelihood of high infection rates [15].

5. Conclusion

HIV infection is an endemic infection in Africa which induces a state of immunosuppression and/or immunocompromised in patients putting them at risk of reactivation of latent and dormant infections. Hence, the prevalence of CMV among HIV-infected individuals is generally low. Since co-infection with CMV accelerates the course of HIV, early therapy is crucial in controlling the spread of the virus. Additionally, sensitization of the population can be done to curb any potential spread of the virus within the community.

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