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Seroprevalence and Molecular Detection of Human Cytomegalovirus in HIV Positive Patients Attending Some Selected Hospitals in Kaduna State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AA designed the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Authors MA, MU and OSO managed the analyses of the study. Author MIT performed the statistical analysis. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The research study aimed to determine the seroprevalence and to molecularly detect cytomegalovirus among HIV patients attending some selected hospitals in Kaduna State, Nigeria. It was also aimed to determine the CD_4^+ cells counts of the HIV positive patients, screen for the presence of CMV IgG and IgM in HIV positive patients using Enzyme Linked Immunosorbent Assay, detect the CMV DNA in IgM-seropositive samples using polymerase chain reaction (PCR), and to determine the predisposing risk factors associated with CMV infection among HIV positive patients using a structured questionnaire.
Place and Duration of Study: The research study was conducted at three selected hospitals located in Kaduna state, Nigeria, between the periods of January, 2015 to May, 2016.

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Methodology: One hundred and seventy eight (178) blood samples were collected from HIV positive patients from the three selected General hospitals in Kaduna state. The blood samples were examined for the presence of CMV IgG and CMV IgM antibodies by ELISA.
Results: A total of 99.4% of HIV infected patients tested positive to CMV IgG antibodies and 11.8% of HIV positive patients tested positive to CMV IgM antibodies. There was no statistically significant difference between human cytomegalovirus infection and CD_4^+ cells range at p = 0.091 for IgG while there was significant difference between HCMV infection at p = 0.039 for IgM. Across geographical zones infection with HCMV showed no significant difference for IgG (χ^2 = 2.081, df = 2, p = 0.353) while significant difference was observed in IgM (χ^2 = 12.808, df = 2, p = 0.002). The prevalence of HCMV DNA among HIV seropositive subject was 45%, out of the eleven randomly selected HIV positive samples glycoprotein B gene of CMV was detected in five of the samples following gel electrophoresis of their PCR amplified product.
Conclusion: The risk factors found to be associated with CMV infection were the geographical location of the HIV positive patients, marital status, and CD_4^+ cells range. There was no statistical association between CMV infection and age, sex, occupation, level of education, multiple sexual partner, blood transfusion, and ART. As a result of the high prevalence of CMV infection observed in this study, it is advisable that patients are tested for specific CMV antibodies in order to identify those at risk of CMV disease that are HIV positive with low CD_4^+ cells count and blood should be properly screened for CMV before transfusion to HIV positive patients.

Keywords: Cytomegalovirus; HIV; IgG; IgM; polymerase chain reaction; seroprevalence.

ABBREVIATIONS

CMV : Cytomegalovirus
HCMV : Human cytomegalovirus
ART : Antiretroviral therapy
ELISA : Enzyme Linked Immuno-sorbent Assay
PMTCT : Prevention of Mother to Child Transmission
MTCT : Mother to Child Transmission
PCR : Polymerase Chain Reaction
HAART : Highly Active Antiretroviral Therapy

1. INTRODUCTION

Human immunodeficiency virus (HIV) continues to be a major global public health issue, having claimed more than 34 million lives so far. People living with HIV have compromised immune systems and are at a much greater risk for serious repercussions from common illnesses and Opportunistic Infections, which are caused by bacteria, viruses, fungi, or protozoa. In 2014, about 1.2 [1.0–1.5] million people died from HIV-related causes globally. There were approximately 36.9 [34.3–41.4] million people living with HIV at the end of 2014 with 2.0 [1.9–2.2] million people becoming newly infected with HIV in 2014 globally [1].

Sub-Saharan Africa is the most affected region, with 25.8 [24.0–28.7] million people living with HIV in 2014. Also sub-Saharan Africa accounts for almost 70% of the global total of new HIV infections [1]. Worldwide, Nigeria has the second highest number of new HIV infections reported each year, and an estimated 3.7 percent of the populations are living with HIV [2,3].

Although HIV prevalence is much lower in Nigeria than in other African countries, such as South Africa and Zambia, the size of Nigeria's population (around 166.6 million) means that by the end of 2011, there were an estimated 3.4 million people living with HIV [2,4]. Approximately 210,000 people died from AIDS in Nigeria in 2011 [2]. In 2012, the national life expectancy was 52 years [3].

Human cytomegalovirus (HCMV) is a species-specific double stranded deoxyribonucleic acid (dsDNA) virus, belonging to the genus herpes virus and family *Herpesviridae*. In humans it is commonly known as human herpes virus 5 (HHV-5). Human cytomegalovirus belongs to the *Betaherpesvirinae* subfamily of *Herpesviridae,* which also includes *Roseolovirus.* Other *herpesviruses* fall into the subfamilies of *Alphaherpesvirinae,* including HSV 1 and 2 and varicella or *Gammaherpesvirinae,* including Epstein-Barr virus. Other HCMV viruses are found in several mammalian species, but species isolated from animals differ from those from humans in terms of genomic structure [5,6].

Cytomegalovirus (CMV) is a ubiquitous herpes virus that generally causes asymptomatic or mildly symptomatic infections in

immunocompetent hosts. In contrast, CMV infection in immunocompromised patients carries high morbidity and mortality [7]. 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 [8,9]. Also immunocompromised hosts could also suffer CMV disease when they are re-infected by different exogenous strains of CMV virus [10]. Cytomegalovirus disease is associated with significantly high morbidity and mortality in the immunocompromised hosts [7]. 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) [11]. Their counterparts who are on HAART tend to have lower incidence of CMV disease [12].

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 [13]. In healthy individuals in the various parts of the world, CMV-IgG seroprevalence ranges from 40-100% [14,15]. Among HIV/AIDS patients, seroprevalences of IgG and IgM ranges from 59.2-100.0% and 0.0-42.9% respectively [11,16].

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 [13,17]. Also CMV may predispose the host to bacterial or fungal infection by compromising the integrity of mucosal barriers to infection. On the other hand tumour necrotic factor (TNF) –α stimulated host cells accumulates intranuclear kB factor leading to activation of CMV DNA replication [13].

Congenital CMV infection has been reported to occur in as high as 23% of neonates born to mothers infected with HIV [18]. Congenital CMV infection has also been shown to be more common in HIV-infected than in HIV-uninfected neonates [18]. This has also been reported to lead to a more rapid progression of HIV infection in these newborns [18]. The association between CMV and HIV infection has been supported by reduction of congenital CMV in areas where prevention of mother to child transmission (PMTCT) of HIV has been implemented. Following the introduction of HAART in developed countries in 1997 to curb the MTCT of HIV infection, there has been a decline in the prevalence of congenital CMV infection [19].

CMV is closely linked to its natural host, human beings. Over the generations, the pathogen and the host have adapted to each other and in most cases, they live in symbiosis. Only in the last few years that scientists succeeded in discovering the strategic weapon which are used by the virus to evade the human immune system [20].

Infection with CMV leads to development of antibodies to the virus that will stay in the body for the rest of that person's life. A blood test of these antibodies can tell whether a person has ever been infected with CMV or not. Serological tests that can indicate when a person was infected are not widely available [21].

Traditionally serologic evidence of recent primary CMV infection depends on the demonstration of conversion from IgG antibody negative to positive or demonstration of IgM antibody [22].

This research study targeted to determine the presence of CMV IgG and IgM in HIV positive patients using ELISA and to detect the CMV DNA in CMV IgM seropositive samples using PCR. The study also aimed to determine the CD4+ cells counts of the HIV positive patients, and to determine the predisposing risk factors associated with CMV infection among HIV positive patients.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Kaduna State, Nigeria. Kaduna State is in the centre of Northern Nigeria and more than 1000 km from the Atlantic Ocean. It is located on a Latitude of 11°12` N and a Longitude of 07°37`E. The State is the successor of the old Northern Region of Nigeria, which had its capital at Kaduna. In 1967 this was divided into Six (6), one of which was the North Central State, which was divided to Kaduna and Katsina State in 1987.

The population of Kaduna State is 6,066,562 [3]. It is characterized by a tropical climate with two seasons; a rainy season of approximately 210 days (May to October), with a mean annual rainfall of 1092.8 mm, and the dry season which spans between November and April. The monthly mean temperature shows a maximum of 39.7℃ in June and 13.8℃ in December.

The State has twenty-three (23) Local Government Areas (LGAs) and three Senatorial Zone. These include the Kaduna north, Kaduna central and Kaduna south Senatorial Zone.

From these three senatorial zones, three hospitals will be selected namely: Barau Dikko Teaching Hospital Kaduna (Kaduna Central), General Hospital Kwoi (Kaduna South), Hajiya Gambo Sawaba General Hospital, Zaria (Kaduna North).

2.2 Study Population

The study population consists of HIV positive patients, both males and females of all ages attending the three selected hospitals, one from each of the three senatorial districts of the state. Participants were asked to and helped to fill the structured questionnaires, which comprised of demographic information, history of previous exposure to blood transfusion and donation, age, socioeconomic background and Sex.

2.3 Inclusion Criteria

The inclusion criteria include consented HIV positive patients of both sexes and ages attending the selected hospitals.

2.4 Exclusion Criteria

The exclusion criteria were non HIV patients and HIV positive patients who did not consent.

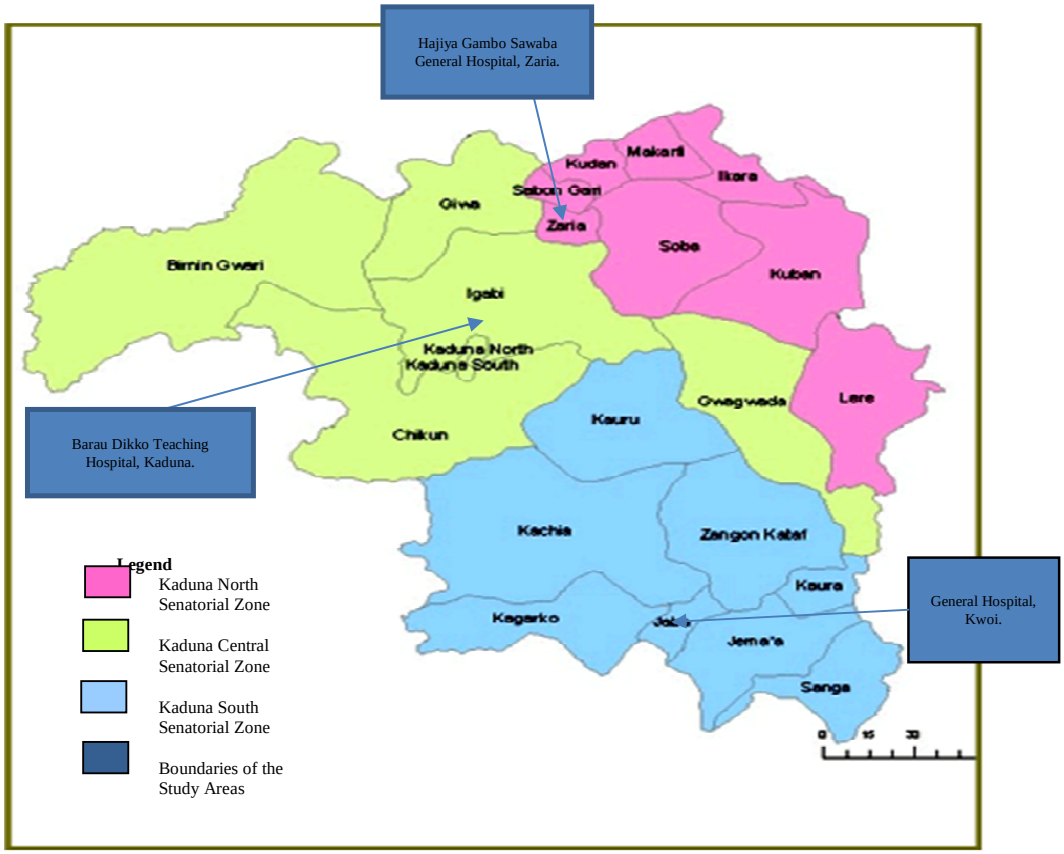

Fig. 1. Administrative Map of Kaduna State showing the study areas, (NPC, 2006)

2.5 Sample Size

The sample size of the study was determined using the prevalence of CMV antibodies of 13.0% in a research conducted at Kano state [23], and the Kish Leisle formula:

$$N=Z^2 P (1-P)/d^2$$

Where

N = sample size
P = prevalence of CMV antibodies =13.0%
Z = standard normal distribution at 95% confidence limit =1.96
D = allowable error (5% = 0.05)

Therefore,

Since

$$N = Z^2 P(1-P)/d^2$$

$$N = \frac{1.962 \times 0.13 \times (1-0.13)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.13 \times 0.87}{0.0025}$$

N= 173.7 samples.

The calculated sample size was 173, hence a total of 178 samples were collected from HIV patients in Kaduna State. 62, 58, 58 samples were collected from the three hospitals respectively.

2.6 Data Collection

Questionnaires were used to collect data on demographic risk factors such as age, town of residence, educational status, occupation, marital status, type of marriage, history of blood transfusion, and history of multiple sexual partners (Appendix III).

2.7 Sample Collection and Processing

A volume of 5ml venous blood samples were collected aseptically (by swabbing with 70% alcohol) with the help of a trained medical personnel from each HIV positive patient and dispensed into an anticoagulant (EDTA) container, between the months of October and December, 2015. The samples were divided into two; one part was used for CD_4^+ cells count estimation, while the other half was centrifuged at 3,000 rpm for 10 minutes. The plasma were separated using pipettes, transferred into sterile cryovials and stored at -20℃ prior to analysis in the Virology Laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria where ELISA technique was carried out. The CD_4^+ cells count analysis was done in the respective hospitals. The PCR testing was done at Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Kaduna, Nigeria.

2.8 Laboratory Diagnosis

Plasma samples were analyzed using Enzyme-linked Immuno-Sorbent Assay (ELISA) technique for IgG and IgM, using the Diagnostic Automation ELISA CMV IgG and IgM kit (Diagnostic Automation, Inc., Woodland Hills, USA).

2.9 Enzyme-linked Immunosorbent Assay

The type of ELISA that was used in the assay was indirect ELISA CMV IgG and IgM kit (Diagnostic Automation, Inc., Woodland Hills, USA). The principle of the assay is that the purified CMV antigens coated on the surface of the micro wells of Diagnostic Automation ELISA IgG or IgM, reacts with CMV specific antibodies by binding (if present in patient's diluted plasma) when added to the wells, all unbound materials are washed away. After the addition of enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time with a stop solution. The intensity of the colour generated is proportional to the amount of IgG or IgM specific antibodies in the samples. The results are read by micro well reader and compared in a parallel manner with calibrators and controls.

2.10 Test Procedure

2.10.1 Enzyme-linked immunosorbent assay (ELISA)

All refrigerated samples and reagent were brought to room temperature (20-25℃) on the laboratory bench and gently mixed. A 1x washing buffer was prepared by adding distilled or deionized water to 20x wash concentrate to a final volume of 1 liter.

A 96 well plane micro titre plate was labelled and positive and negative controls, cut off calibrator, positive and negative calibrators and samples were labelled according to labels of the wells in the micro titre plate. A 1:40 dilution of samples, control positive and negative cut off calibrator and negative and positive calibrators were prepared by pipetting 195 µl of sample diluent using 200 µl multiple channeled micropipettes into each labelled well. About 5 µl of the test samples, negative and positive controls, cut off and calibrators were pipetted using 10 µl micropipette into the 195 µl of sample diluents in the corresponding wells. The micro titre plate was then shaken gently on the surface of the bench to mix the contents.

A 96 well micro titre plate coated with CMV antigens in each well, was labelled the same way the plane micro titre plate was labelled and 100 µl of diluted test samples, calibrators, cut off calibrator and positive and negative controls were pipetted using 200 µl multiple channeled micropipette and dispensed into corresponding labelled wells of the CMV antigens coated micro titre plate. A volume of 100 µl of sample diluent was dispensed into 1A well position as blank. The holder was tapped to remove air bubbles from the liquid and mixed well followed by incubation for 30 minutes at room temperature.

The diluted samples, positive and negative controls, cut off, calibrators and blank were all removed from wells and washed three times with washing buffer then blotted with tissue paper.

A volume of 100 µl of enzyme conjugate was pipetted using multiple channeled micropipettes and dispensed into each well and incubated for 30 minutes at room temperature after which the enzyme conjugate was removed from all the wells and washed three times with wash buffer as before followed by blotting with tissue paper.

About 100 µl of TMB chromogenic substrate was pipetted with multiple channeled micropipettes and dispensed into each well then incubated for 15 minutes at room temperature.

Finally, 100 µl of stop solution was added with the use of multiple channeled micropipettes to each well to stop the reaction, making sure that there were no air bubbles before reading.

The optical density (O.D) was then read with GF-3000 micro plate reader (B brand scientific instrument England) at 450 nm wavelength.

2.11 Results for CMV IgG

1. The mean of duplicate calibrator value x_c was calculated.
2. The mean of duplicate positive control, negative control and patient samples were calculated.
3. The CMV G Index of each determination was calculated by dividing the mean values of each sample by calibrator mean value, x_c.

2.12 Interpretation

Negative: CMV G Index of 0.90 or less are seronegative for IgG antibody to CMV. (<1.1 IU/ml)

Equivocal: CMV G Index of 0.91-0.99 are equivocal.

Positive: CMV G Index of 1.00 or greater, or IU value greater than 1.2 are seropositive.

2.13 Results for CMV IgM

1. To obtain cut off OD value: The OD of calibrator was multiplied by factor (f) printed on label of calibrator.
2. The CMV IgM Index of each determination was calculated by dividing the OD value of each sample by obtained OD value of cut off.

2.14 Interpretation

Negative: CMV M Index of less than 0.09 are negative for IgM antibody to CMV.

Equivocal: CMV M Index of less than 0.91-0.99 is equivocal.

Positive: CMV M Index of 1.0 or greater are positive for IgM antibody to CMV.

2.15 CD_4^+ Cells Estimation

The CD_4^+ was determined using an automated Cyflow CD_4^+ cells counter. Twenty microlitre (20 ul) CD_4^+ (CD_4^+ MAB PE PARTEC CD_4^+) antibody was pipetted into Partec test tube (Rohren tube) followed by twenty microlitre (20 ul) of well mixed whole blood, was gently mixed and incubated in the dark for 15 minutes at room temperature. Eight hundred microliter (800 µl) of CD_4^+ buffer (Essy count kit no lais PARTEC CD_4^+) was

pipetted into the test tube and also mixed gently. The tube was then plugged on the sensor for counting as adopted by Musa et al. [23].

2.16 Molecular Detection of CMV

The detection of CMV DNA in IgM-seropositive samples was done by Polymerase Chain Reaction (PCR).

2.17 PCR Assay

Only samples that are seropositive to CMV IgM antibody were subjected to PCR testing. Cytomegalovirus DNA was detected by PCR assay using pair of primers associated with the glycoprotein B gene the size of the B gene is 257bp: 5'-CGGTGGAGATACTGCTGAGGTC-3'(P1, sense nucleotides 82494-82515), 5'-CAAGGTGCTGCGTGATATGAAC-3'(P2, antisense nucleotides 82729-82750) [24].

2.18 Genomic DNA Extraction Using Zymo Research Kits

One (1) ml of the sample was dispensed to each 1.5 ml eppendorff tubes, the tubes were centrifuged at 10000 g for 1 min, supernatant was discarded, and Pellets were re-suspended in 600 µl lysis buffer and incubated at 56℃ for 30 mins. The mixture was transferred to spin column in a collection tubes and centrifuged at 10000 g for 1min, flow through together with the column was discarded.

The spin column thereafter was inserted into a new collection tubes 500 µl of wash buffer I was added and centrifuged at 10000 g for 1 min, flow through was discarded, 500 µl of was buffer II was also added and centrifuged at 10000 g for 1 min, the flow through was discarded.

The spin column was then centrifuged at 10000 g for 2 mins to remove residual alcohols and salt, the collection tube was discarded and the spin column inserted into a 1.5 ml micro-centrifuge eppendorff tube. One hundred (100) ml of elution buffer was added to the spin column and incubated at room temperature for 2mins, and centrifuged at 10000 g for 2 mins, and then the spin column was discarded.

The resultant DNA in 1.5 ml micro-centrifuge tube was stored in -86℃ refrigerator for later use in the downstream applications.

2.19 Electrophoresis

The casting apparatus was assembled with the combs placed in position, 1.5 g of Agarose powder was weighed and 100 ml of TBE buffer was added, the mixture was microwaved and cooled to 45℃. Five (5 µl) ethidium bromide was added and shaken to be uniformly distributed, the preparation was carefully poured into the casting tray and allowed to solidify, the combs were carefully removed and the casting tray was removed and transported to electrophoretic tank containing TBE buffer, the vacuum of the buffer was adjusted to the maximum.

Fifteen (15) µl of PCR product was mixed with 5 µl of 6 times loading buffer and loaded into each well, the first well was loaded with 50 bp DNA ladder, the tank was covered and connected to electric power pack, which was switched on and ran at 80V for 45 min.

The gel tray was taken into gel documentation units, the gel was carefully slipped down from the tray to the gel documentation system, and the gel was viewed and documented in a computer system [24].

2.20 Statistical Analysis

The data obtained from the questionnaire and the results of the laboratory analysis were entered into SPSS (statistical package for social sciences Version 20) and analyzed. The results obtained were reduced to percentages and figures. The Pearson Chi square test at 95% confidence interval at 0.05 level of significance was used to determine the relationship between the demographic data and prevalence rates.

3. RESULTS

3.1 Seroprevalence Outcome of HIV Positive Patients Tested for Anti-CMV IgG/IgM Antibodies among the Selected Hospitals in Kaduna State

A total of 178 blood samples of HIV positive patients attending the three selected General Hospitals in Kaduna State were randomly collected. Volunteers were drawn from Hajiya Gambo Sawaba General Hospital Zaria (HGSGHZ), General Hospital Kwoi (GHK), and Barau Dikko Teaching Hospital Kaduna (BDTHK). Each hospital was represented with 62, 58, 58 patients respectively.

Questionnaires were administered to these patients to obtain information used to assess the risk factors associated to CMV infection.

The overall seroprevalence of CMV IgG and IgM among the studied population was found to be 99.4% (177/178) and 11.8% (21/178) respectively. The result distributed by hospitals has shown the seroprevalence of 100% (62/62), 98.3% (57/58), 100% (58/58) for IgG and 0% (0/62), 18.9% (11/58), 17.2% (10/58) for IgM from the three hospitals; HGSGHZ, GHK, BDTHK respectively. The seroprevalence of IgG showed no significant difference across the three hospitals studied $\chi^2 = 2.081$, df = 2, p = 0.353). While significant difference was observed in IgM between the three hospitals ($\chi^2 = 12.808$, df = 2, p = 0.002), (Table 1).

3.2 Seroprevalence Outcome of HCMV in Relation to CD_4^+ Cells Range

The prevalence of HCMV base on CD_4^+ cells range in this study showed no significant difference between CD_4^+ cells range and HCMV infection at p = 0.091 for IgG while there was significant difference between CD_4^+ cells range and HCMV infection at p = 0.039 for IgM. The highest prevalence was recorded in CD_4^+ cells range 251-500 which was 78(43.8%) for IgG and 501-750 which is 7(3.9%) for IgM, with the lowest prevalence recorded in CD_4^+ cells range 1001-1250 which is 1(0.6%) for IgG and 1501-1750 which is 0(0.0%) for IgM, (Fig. 2).

3.3 Comparative Seropositivity of HCMV IgG/IgM among HIV Seropositive Patients

Out of the 178 samples analyzed, 177 were positive to IgG whereas only one sample was found to be negative to IgG. While 21 were positive to IgM and 157 were negative to IgM as shown in figure (Fig. 3).

3.4 Socio-demographic Data and Seropositive Outcome of HIV Patients Tested for Anti-CMV IgG/IgM Antibodies

From the various socio-demographic data for HCMV infection considered, age was classified into fifteen (15) different age groups of five (5) years interval as follows: 1-5, 6-10, to 71-75. The result shows that there was no significant difference between age group and HCMV infection in both IgG and IgM at p = 0.994 and p = 0.656 respectively. Highest prevalence was observed in HIV patient in age group 36-40 with 34(19.1%) for IgG, while in age group 31-35 with 8(4.8%) for IgM. On the other hand lowest prevalence was observed in the age group 1-5 with 1(0.6%) for IgG and 71-75 with 0(0.0%) for IgM.

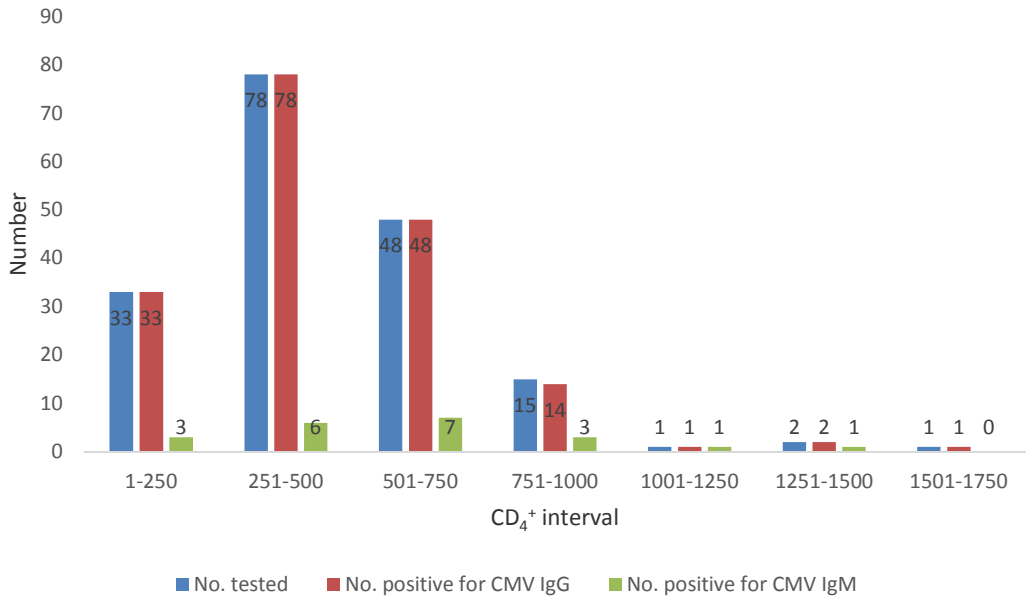

Fig. 2. Seropositivity of CMV antibodies in relation to $CD4^+$ counts among HIV patients

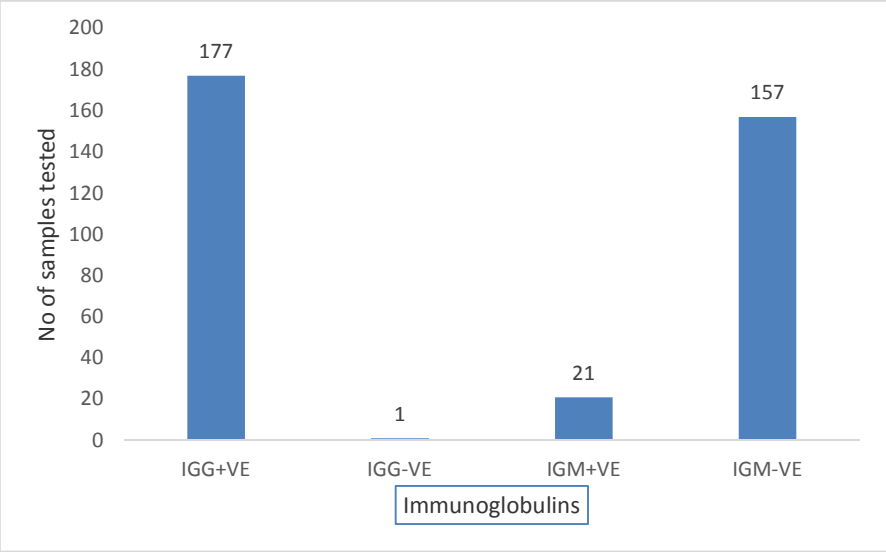

Fig. 3. Comparison of CMV IgM and CMV IgG seropositivity among HIV patients

Table 1. Seroprevalence outcome of HIV patients tested for anti-CMV IgG/IgM antibodies among the selected hospitals in Kaduna State

Variables	No. tested (%)	No. positive (%) anti-CMV IgG	Statistical values	No. positive (%) anti-CMV IgM	Statistical values
Hospital					
HGSGHZ	62(34.8)	62(100)	$\chi^2 = 2.081$	0(0.0)	$\chi^2 = 12.808$
GHK	58(32.6)	57(98.3)	df = 2	11(19.0)	df = 2
BDTH	58(32.6)	58(100)	P = 0.353	10(17.2)	P = 0.002
Total	178(100)	177(99.4)		21(11.8)	

HGSGHZ = Hajiya Gambo Sawaba General Hospital Zaria; GHK = General Hospital Kwoi; BDTHK = Barau Dikko Teaching Hospital Kaduna

Analysis of prevalence base on sex shows that there was no significant difference between HCMV and sex at p = 0.497 and p = 0.071 for IgG and IgM respectively. With female having the highest prevalence of 121(67.9%) and 18(10.1%) IgG and IgM respectively. While male have the lowest prevalence of 56(31.5) and 3(1.7) IgG and IgM respectively.

The prevalence base on marital status showed that there was no significant difference between HCMV infection and marital status for IgG at p = 0.895, but there was significant difference between HCMV infection and marital status for IgM at p = 0.020. With married having the highest prevalence of 110 (61.8%) and 11(6.2%) for IgG and IgM respectively, while the lowest prevalence was observed in divorced 9(5.1%) for IgG and widow 2(1.1%) for IgM.

Further, analysis of HCMV infection base on type of marriage, showed that there was no significant difference between type of marriage and HCMV infection at p = 0.722 and p = 0.253 for IgG and IgM respectively. However, highest prevalence was recorded in monogamy 107(60.1%) and 16(9.0%) for IgG and IgM respectively with the lowest prevalence in not application 31(17.4%) and 3(1.7%) IgG and IgM respectively.

The prevalence of HCMV base on occupation showed that there was no significant difference between occupation and HCMV infection at p = 0.411 and p = 0.330 for IgG and IgM respectively. Highest prevalence was recorded in house wife 62(34.8%) and 7(3.9%) for IgG and IgM respectively, with the lowest prevalence recorded in student 9(5.1%) and 0(0.0%) for IgG and IgM respectively.

The prevalence of HCMV base on level of education showed that there was no significant difference between level of education and HCMV infection at p = 0.721 and p = 0.500

for IgG and IgM respectively. With the highest prevalence recorded in secondary 57(32.0%) and 8(4.5%) for IgG and IgM respectively, lowest prevalence was observed in not educated 12(6.7%) for IgG and in tertiary 3(1.7%) for IgM.

Analysis of HCMV infection among household number range showed that there was no significant difference between household number range and HCMV infection at p = 0.903 and p = 0.573 for IgG and IgM respectively. Highest prevalence was recorded in household number range 1-5 which is 89(50.0%) for IgG and 6-10 which is 11(6.2%) for IgM, with the lowest prevalence recorded in household number range 21-25 which is 2(1.1%) and 0(0.0%) for IgG and IgM respectively (Table 2).

Table 2. Socio-demographic data and seropositive outcome of HIV patients tested for anti-CMV IgG/IgM antibodies

Variables	No. tested (%)	No. positive (%) anti-CMV IgG	Statistical values	No. positive (%) anti-CMV IgM	Statistical values
Age(years)					
1-5	1(0.6)	1(0.6)		0(0.0)	
6-10	1(0.6)	1(0.6)		0(0.0)	
11-15	1(0.6)	1(0.6)		0(0.0)	
16-20	4(2.2)	4(2.2)		1(25)	
21-25	10(5.6)	10(5.6)	$\chi^2 = 4.259$	2(20)	$\chi^2 = 11.385$
26-30	34(19.1)	33(18.5)	df = 14	2(5.8)	df = 14
31-35	33(18.5)	33(18.5)	P = 0.994	8(24)	P = 0.656
36-40	34(19.1)	34(19.1)		3(8.8)	
41-45	21(11.8)	21(11.8)		2(9.5)	
46-50	18(10.1)	18(10.1)		3(16.7)	
51-55	13(7.3)	13(7.3)		0(0.0)	
56-60	4(2.2)	4(2.2)		0(0.0)	
61-65	1(0.6)	1(0.6)		0(0.0)	
66-70	1(0.6)	1(0.6)		0(0.0)	
71-75	2(1.1)	2(1.1)		0(0.0)	
Total	178(100)	177(99.4)		21(11.8)	
Sex					
Male	56(31.5)	56(31.5)	$\chi^2 = 0.462$ df = 1	3(5.4)	$\chi^2 = 3.257$ df = 1
Female	122(68.5)	121(67.9)	P = 0.497	18(14.8)	P = 0.071
Total	178(100)	177(99.4)		21(11.8)	
Marital status					
Single	34(19.1)	34(19.1)		4(11.8)	
Married	111(62.4)	110(61.8)		11(10)	$\chi^2 = 9.875$
Divorced	9(5.1)	9(5.1)	$\chi^2 = 0.607$	4(44.4)	df = 3
Widow	24(13.5)	24(13.5)	df = 3	2(8.3)	P = 0.020
Total	178(100.1)	177(99.5)	P = 0.895	21(11.8)	
Types of marriage					
Monogamy	108(60.7)	107(60.1)	$\chi^2 = 0.652$	16(9.0)	$\chi^2 = 2.746$
Polygamy	39(21.9)	39(21.9)	df = 2	2(1.1)	df = 2
Not Applicable	31(17.4)	31(17.4)	P = 0.722	3(1.7)	P = 0.253
Total	178(100)	177(99.4)		21(11.8)	
Occupation					
Civil Servant	37(20.8)	37(20.8)		2(1.1)	
Farmer	34(19.1)	34(19.1)	$\chi^2 = 3.967$	6(3.4)	$\chi^2 = 4.610$
Business	36(20.2)	35(19.7)	df = 4	6(3.4)	df = 4
House Wife	62(34.8)	62(34.8)	P = 0.411	7(3.9)	P = 0.330

Variables	No. tested (%)	No. positive (%) anti-CMV IgG	Statistical values	No. positive (%) anti-CMV IgM	Statistical values
Student	9(5.1)	9(5.1)		0(0.0)	
Total	178(100)	177(99.5)		21(11.8)	
Level of education					
Primary	32(18.0)	32(18.0)		4(2.2)	
Secondary	58(32.6)	57(32.0)	$\chi^2 = 2.081$	8(4.5)	$\chi^2 = 3.360$
Tertiary	38(21.3)	38(21.3)	df = 4	3(1.7)	df = 4
Quranic	38(21.3)	38(21.3)	P = 0.721	3(1.7)	P = 0.500
Not Educated	12(6.7)	12(6.7)		3(1.7)	
Total	178(99.9)	177(99.3)		21(11.8)	
Household no. range					
1-5	89(50.0)	89(50.0)		10(5.6)	
6-10	69(38.8)	68(38.2)		11(6.2)	
11-15	11(6.2)	11(6.2)	$\chi^2 = 1.589$	0(0.0)	$\chi^2 = 3.841$
16-20	5(2.8)	5(2.8)	df = 5	0(0.0)	df = 5
21-25	2(1.1)	2(1.1)	P = 0.903	0(0.0)	P = 0.573
26-30	2(1.1)	2(1.1)		0(0.0)	
Total	178(100)	177(99.4)		21(11.8)	

3.5 Risk Factors and Seropositive Outcome of HIV Patients Tested for Anti-CMV IgG/IgM Antibodies

Analysis of HCMV infection base on preferred mode of sexual activity showed that there was no significant difference between preferred mode of sexual activity and HCMV infection at p = 0.940 and p = 0.714 for IgG and IgM respectively.

Analysis in relation to multiple sexual partner with HCMV, showed that there was no significant difference between multiple sexual partner and HCMV infection at p = 0.678 and p = 0.540 for IgG and IgM respectively.

In relation to tribal mark with HCMV, showed that there was no significant difference between tribal mark and HCMV infection at p = 0.359 and p = 0.108 for IgG and IgM respectively.

Analysis in relation to hard drug with HCMV, showed that there was no significant difference between hard drug and HCMV infection at p = 0.865 and p = 0.407 for IgG and IgM respectively. Analysis base on blood transfusion with HCMV, showed that there was no significant difference between blood transfusion and HCMV infection at p = 0.737 and p = 0.387 for IgG and IgM respectively.

Analysis base on ART with HCMV, showed that there was no significant difference between ART and HCMV infection at p = 0.797 and p = 0.498 for IgG and IgM respectively, (Table 3).

3.6 Clinical Features and Seropositive Outcome of HIV Patients Tested for Anti-CMV IgG/IgM Antibodies

Further analysis of the result based on clinical features, history of temporary deafness or partial blindness with HCMV showed that there was no significant difference between history of temporary deafness or partial blindness and HCMV infection at p = 0.114 and p = 0.308 for IgG and IgM respectively.

Analysis in relation to spontaneous miscarriage with HCMV, showed that there was no significant difference between spontaneous miscarriage and HCMV infection at p = 0.555 and p = 0.099 for IgG and IgM respectively.

Analysis in relation to fever with HCMV, showed that there was no significant difference between fever and HCMV infection at p = 0.349 and p = 0.304 for IgG and IgM respectively.

Analysis in relation to fatigue with HCMV, showed that there was no significant difference between fatigue and HCMV infection at p = 0.349 and p = 0.923 for IgG and IgM respectively. Analysis in relation to headache with HCMV, showed that there was no significant difference between headache and HCMV infection at p = 0.419 and p = 0.549 for IgG and IgM respectively. Analysis in relation to difficulty in breathing with HCMV, showed that there was no significant difference between difficulty in breathing and HCMV infection at p = 0.632 and p = 0.949 for IgG and IgM respectively.

Table 3. Risk factors and seropositive outcome of HIV patients tested for anti-CMV IgG/IgM antibodies

Variables	No. tested (%)	No. positive anti-CMV IgG (%)	Statistical values	No. positive anti-CMV IgM (%)	Statistical values
CD4+ cells range					
1-250	33(18.5)	33(18.5)		3(1.7)	
251-500	78(43.8)	78(43.8)		6(3.4)	
501-750	48(27.0)	48(27.0)	χ^2 = 10.928	7(3.9)	χ^2=13.238
751-1000	15(8.4)	14(7.9)	df = 6	3(1.7)	df = 6
1001-1250	1(0.6)	1(0.6)	P = 0.091	1(0.6)	P = 0.039
1251-1500	2(1.1)	2(1.1)		1(0.6)	
1501-1750	1(0.6)	1(0.6)		0(0.0)	
Total	178(100)	177(99.5)		21(11.9)	
Preferred mode of sexual activity					
Homosexual	1(0.6)	1(0.6)	χ^2 = 0.006	0(0.0)	χ^2 = 0.135
Heterosexual	177(99.4)	176(98.8)	df = 1	21(11.8)	df = 1
Total	178(100)	177(99.4)	P = 0.940	21(11.8)	P = 0.714
Multiple sexual partner					
Yes	26(14.6)	26(14.6)	χ^2 = 0.172	4(2.2)	χ^2 = 0.376
No	152(85.4)	151(84.8)	df = 1	17(9.6)	df = 1
Total	178(100)	177(99.4)	P = 0.678	21(11.8)	P = 0.540
Tribal mark					
Yes	97(54.5)	96(53.9)	χ^2 = 0.840	8(4.5)	χ^2 = 2.582
No	81(45.5)	81(45.5)	df = 1	13(7.3)	df = 1
Total	178(100)	177(99.4)	P = 0.359	21(11.8)	P = 0.108
Hard drug					
Yes	5(2.8)	5(2.8)	χ^2 = 0.029	0(0.0)	χ^2 = 0.688
No	173(97.2)	172(96.6)	df = 1	21(11.8)	df = 1
Total	178(100)	177(99.4)	P = 0.865	21(11.8)	P = 0.407
Blood transfusion					
Yes	18(10.1)	18(10.1)	χ^2 = 0.113	1(0.6)	χ^2 = 0.750
No	160(89.9)	159(89.3)	df = 1	20(11.2)	df = 1
Total	178(100)	177(99.4)	P = 0.737	21(11.8)	P = 0.387
On ART					
Yes	167(93.8)	166(93.2)	χ^2 = 0.066	19(10.7)	χ^2 = 0.459
No	11(6.2)	11(6.2)	df = 1	2(1.1)	df = 1
Total	178(100)	177(99.4)	P = 0.797	21(11.8)	P = 0.498

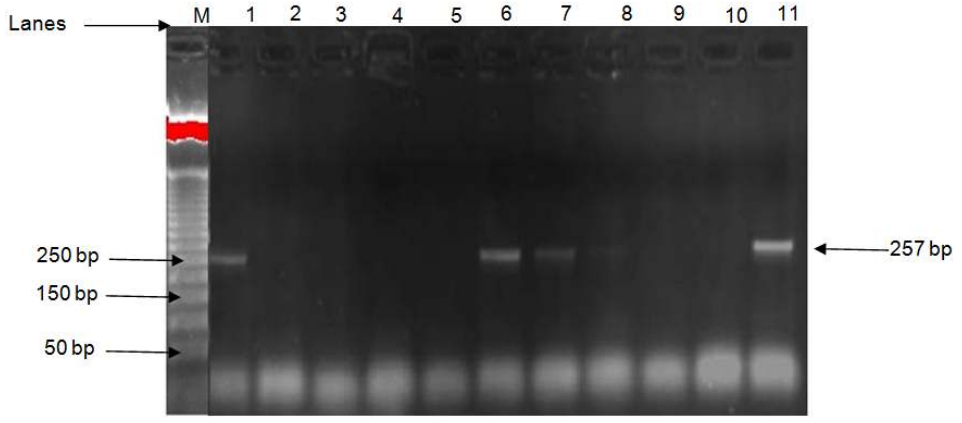

Plate I. Agarose gel electrophoresis of PCR amplified glycoprotein B gene of cytomegalovirus: Lane M 50 bp DNA ladder and lanes 1-11 PCR amplicons

Table 4. Clinical features and seropositive outcome of HIV patients tested for anti-CMV IgG/IgM antibodies

Variables	No. tested (%)	No. positive (%) anti-CMV IgG	Statistical values	No. positive (%) anti-CMV IgM	Statistical values
History of temporary deafness or partial blindness					
Yes	51(28.7)	50(28.1)	$\chi^2 = 2.504$	8(4.5)	$\chi^2 = 1.039$
No	127(71.3)	127(71.3)	df = 1	13(7.3)	df = 1
Total	178(100)	177(99.4)	P = 0.114	21(11.8)	P = 0.308
Spontaneous miscarriage					
Yes	39(21.9)	39(21.9)	$\chi^2 = 1.177$	4(2.2)	$\chi^2 = 4.621$
No	82(46.1)	81(45.5)	df = 2	14(7.9)	df = 2
Not applicable	57(32.0)	57(32.0)	P = 0.555	3(1.7)	P = 0.099
Total	178(100)	177(99.4)		21(11.8)	
Fever					
Yes	95(53.4)	94(52.8)	$\chi^2 = 0.879$	9(5.1)	$\chi^2 = 1.058$
No	83(46.6)	83(46.6)	df = 1	12(6.7)	df = 1
Total	178(100)	177(99.4)	P = 0.349	21(11.8)	P = 0.304
Fatigue					
Yes	83(46.6)	83(46.6	$\chi^2 = 0.879$	10(5.6)	$\chi^2 = 0.009$
No	95(53.4)	94(53.4)	df = 1	11(6.2)	df = 1
Total	178(100)	177(100)	P = 0.349	21(11.8)	P = 0.923
Headache					
Yes	108(60.7)	107(60.1)	$\chi^2 = 0.652$	14(7.9)	$\chi^2 = 0.358$
No	70(39.3)	70(39.3)	df = 1	7(3.9)	df = 1
Total	178(100)	177(99.4)	P = 0.419	21(11.8)	P =0.549
Difficulty in breathing					
Yes	33(18.5)	33(18.5)	$\chi^2 = 0.229$	4(2.2)	$\chi^2 = 0.004$
No	145(81.5)	144(80.9)	df = 1	17(9.6)	df = 1
Total	178(100)	177(99.4)	P = 0.632	21(11.8)	P = 0.949
Nose bleeding					
Yes	14(7.9)	13(7.3)	$\chi^2 = 11.780$	1(0.6)	χ^2=0.316
No	164(92.1)	164(92.1)	df = 1	20(11.2)	df = 1
Total	178(100)	177(99.4)	P = 0.001	21(11.8)	P = 0.574

Analysis in relation to nose bleeding with HCMV, showed that there was significant difference between nose bleeding and HCMV infection at p = 0.001 for IgG and there was no significance difference at p = 0.574 for IgM, (Table 4).

3.7 Polymerase Chain Reaction (PCR)

Out of the eleven randomly selected HCMV IgM positive samples used, glycoprotein B gene of cytomegalovirus was detected in five of the samples following agarose gel electrophoresis of their PCR amplified product. The band produced was at 250bp corresponding to the amplicon size of the glycoprotein B gene of cytomegalovirus. Therefore, the seroprevalence of CMV DNA among HIV seropositive subjects was 45% (Plate I).

4. DISCUSSION

Analysis of the result base on CD_4^+ cells count shows that there was no significant association between HCMV infection and CD_4^+ cells count for IgG, which agrees with the findings of Irna et al. [25]. But for IgM there was significant association between HCMV and CD_4^+ cells count which agrees with the findings of Adeola et al. [26], but contrary to the findings of Basawaraju et al. [13]; Ojide et al. [27] and Musa et al. [23] in which there is no association. This variation might be due to the fact that most of the HIV positive patients enrolled in this study were on HAART which boost their immune system with their CD_4^+ cells count above 300/ µl. The reason for this is not clear but since most of the patients are on HAART which affects CD_4^+ cell counts, factors

like duration and type of therapy may acts as cofounders.

The results obtained from this study showed that the seroprevalence rate of HCMV infection among HIV positive patients in Kaduna State is high. A seroprevalence of HCMV, 99.4% for IgG and 11.8% for IgM, of the cytomegalovirus infection among HIV patients in Kaduna State was obtained in this study. This signify that HIV is a predisposing factor for HCMV. This result is similar to the seroprevalence of 100% among HIV positive patients reported in Maiduguri University Teaching Hospital north east Nigeria, and from Lagos University Teaching Hospital, south west Nigeria, and Burkina Faso by Ibrahim et al. [28]; Akinbami et al. [29] and Ledru et al. [30], respectively that documented a prevalence of anti-CMV IgG antibody of 100% among HIV-infected patients. It also agrees with the findings of Yeroh et al. [31] who reported the prevalence of 94.8% among pregnant women in Kaduna State. It also agrees with the work of Ojide et al. [27] who reported the prevalence of 98.8% for IgG.

However, this is contrary to the report by Manjusha et al. [32] stating a lower seroprevalence of CMV- IgG (10.4%) and CMV - IgM (8.4%) antibody in HIV positive patients.

In this study, the specific CMV IgM antibodies seropositivity was found to be 21(11.8%). This seroprevalence is high when compared to the prevalence range of (8.0-8.5%) in developing countries [33,34]. It is equally higher than the world range (0 – 10%) [35]. The prevalence rate is higher when compared to the work done by Akinbami et al. [36] (6.6%) in their study among immunocompromised (HIV) patients at Lagos University Teaching Hospital. It is also high when compared to the work of Basawaraju et al. [13] (9.52%) that was carried out among AIDS patients in India. The prevalence rate does not correlate with those of Chakravarti et al. [17]; Chakravarti et al. [37]; and Mujtaba et al. [38] in which they observed seropositivity rates of about 3 – 10%. Our finding is almost similar to the work done by Neusa et al. [39] in Brazil among HIV infected prison inmates where prevalent rate of 11.36% was reported. It is also similar when compared to the work done by Adeola et al. [40] in Ilorin with the prevalence of 11.1%. It is however lower, when compared to the work done by Musa et al. [23] in Kano with prevalent rate of 13%, and [41] who reported prevalent rate of 14% among HIV patients. The reason for the high prevalence of HCMV infection among HIV positive patients in Kaduna State may be associated with the depreciating socioeconomic standard, poor hygienic practices, low standard of education among the rural patients. Racial differences between the populations, enormous cultural and economic differences between developed countries (where the study was previously carried out) and developing countries like Nigeria are valid factors that might be responsible for this variation in prevalence rates obtained, which justifies the report of Neto et al. [42] and Nishimura et al. [43].

The distribution of the prevalence rates of HCMV infection in the three geographical zones, for IgG shows that there was no significant difference with Zaria having the highest prevalence rate and Kwoi having the lowest prevalence rate.

Most of the HIV positive patients involved in this study from Zaria zone, came from Zaria city where there is congestion of settlement leading to poor hygienic living and overcrowding. It was also noticed that majority of the HIV positive patients from Zaria zone have low education and are non-employed which could possibly be the reasons of this highest prevalence [31]. The lowest prevalence rate obtained in Kwoi might be as a result of enlightenment and better hygienic environments for living. This result agrees with the report of [44] who asserted that seroprevalence of HCMV differs by countries and regions. But on the other hand, for the seroprevalence of IgM in the study areas, there was a significant difference between the three geographical zones, with Kwoi having the highest prevalence rate and Zaria having the lowest prevalence rate.

The prevalence of HCMV DNA among HIV seropositive subjects was found to be 45%. This gene can be detected as early as one week after initial infection, the individuals harboring the active virus could progress to chronic infection and eventually develop ocular retinitis and encephalitis if not treated.

Analysis of the results by age shows that there was no significant association between CMV and age, which agrees with the report of Yeroh et al. [31] and contrary to previous report of Okwori et al. [45] and Adeola et al. [40].

Seropositivity of specific CMV IgM antibodies in relation to different age groups revealed that CMV antibodies are high between age groups 21–30 and 31–40 years. This finding agrees with that of Musa et al. [23] and also is in

concordance with the study conducted by Okwori et al. [45]. Zhong and Ma [46] reported that this trend could be attributed to the fact that the above mentioned age groups represent active and sexually matured youths with the tendency towards sexual promiscuity and its resultant likelihood of high infection rates.

There was no association between HIV positive patients who received blood transfusion and those that were not, and the seroprevalence was higher among those that were not transfused than those that had been transfused, implying that previous history of blood transfusion is not a risk factor for CMV infection. This agrees with the findings of [15,31,40]. But disagrees with the report of [47], where blood transfusion was shown to be a risk factor for transmission of CMV infection. The reasons for the disagreement here could be as a result of the disproportionate size of HIV positive patients who were transfused to those who were not transfused enrolled in the study, and possible explanations is that there are various other routes through which CMV is transmitted in Nigeria including intrauterine (or at parturition), breastfeeding, sexual contact and spread from children.

There was no significant difference in the level of CMV IgM among the HIV positive patients that were on ART and those that were not. This agrees with the findings of Adeola et al. [40].

Analysis of the result base on preferred mode of sexual activity showed that there was no significant difference between CMV IgG infection and preferred mode of sexual activity, with the prevalence of 99.8% for heterosexual patients. This closely agrees with the findings of Ngangom et al. [48] who reported the prevalence of 96.8%. Also, a study that was carried out in Ghana by Compston et al. [49], found that more than 90% of the heterosexually acquired HIV infected persons are positive for CMV IgG.

Our study on the multiple sexual partners in the study area showed that there was no significant difference between the CMV IgG and IgM infection and multiple sexual partners. This deviates from the findings of [40], who reported that there is statistical association in the seropositivity outcome of anti-CMV IgM and anti-CMV IgG antibodies and the number of sexual partners. The disagreement might be due to the fact that there are other routes of acquiring CMV if in contact with saliva, urine, semen of an infected person.

There was no significant difference in the seropositivity outcome of anti-CMV IgM and IgG antibody among the two sex groups, which agrees with the findings of [40]. But, there was deviation from what was reported by Redwan et al. [50], in which there was significant difference. This study revealed that females living with HIV are more susceptible to specific CMV IgM and IgG antibodies than their male counterparts; female subjects showed high prevalence rate of 18 (10.1%) IgM and 121(67.9%) IgG than males 3 (1.7%) IgM and 56 (31.5%) IgG. The infective/reactive rate was significantly higher among the females than in their males' counterparts. The possible reason is that their immune system is suppressed during mensuration, pregnancy and poor hygienic practice.

Based on level of education, our study showed that there was no significant difference between CMV IgG and IgM infection and level of education. This agrees with the findings of [51]. It also agrees with the findings of [31,52].

This study shows no statistical association in the seropositivity outcome of anti-CMV IgM antibody among the various occupational groups. Also, it was observed that there was no significant difference in the seropositivity outcome of anti-CMV IgG antibodies among the subjects. This agrees with the findings of [40,51,52].

Based on marital status, our study showed that there was no significant difference between CMV IgG infection and marital status but significance difference was observed between CMV IgM infection and marital status which is in line with the findings of [40,51]. The seroprevalence rate is higher among the married perhaps due to the direct contact with contagious secretions from their own children or poor hygiene practice. Based on the use of hard drugs, the findings showed that there was no significant difference between CMV infection and use of hard drugs in the study area. This agrees with the findings of [52]. Whereas, based on spontaneous miscarriage, it was found that there was no significant difference between CMV infection and spontaneous miscarriage, which agrees with the findings of Umeh et al. [52].

5. CONCLUSION

This study has demonstrated the prevalence of cytomegalovirus among HIV positive patients in Kaduna State, Nigeria. The data were generated from three Hospitals, one from each of the three

senatorial region of the state. Our study provides evidence that cytomegalovirus infection is highly endemic in the studied area, thereby posing a threat of hearing loss, neurodevelopmental delay and vision loss. Thus, HIV and HCMV mixed infections were recorded in the study area. With this high prevalence rate, lack of vaccine against the disease, and traditional lifestyle that predisposes acquiring infection, transmission of the virus will probably increase and pose more potential public health concern.

Detection of CMV by PCR among HIV positive patients indicates need for therapeutic intervention and other baseline laboratory investigation to establish prognosis and also for use as benchmark for the treatment of the disease.

The risk factors that were found to be associated with CMV infection in the study area include; geographical location of the HIV positive patients, marital status, and CD_4^+ cells range. There was no statistical association between CMV infection and age, sex, occupation, level of education, multiple sexual partner, and preferred mode of sexual activity, tribal mark, hard drug, blood transfusion and ART.

6. RECOMMENDATION

The populace needs to be engaged in health talk creating awareness of the infection and its consequences. This knowledge will help in curbing the transmission. This campaign will stress issues like dangers of indiscriminate unprotected sex, traditional scarification and tattooing, transmission from mother to child and other crude practices that predispose infection.

Since almost all subjects were exposed to CMV infection, intervention should be step up to prevent vertical transmission of human CMV from HIV infected mothers to their children.

There is need for partnership between non-governmental organizations and government to intervene on issues of vaccine development, treatment and surveillance.

CONSENT

All authors declare that written informed consent was obtained from the patients (Appendix II), and the authority of Ahmadu Bello University, Zaria (Appendix I), for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the Research Ethical Committee of the Ministry of Health, Kaduna State, Nigeria, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. HIV/AIDS fact sheet; 2015.
   Available:http://www.who.int/mediacentre/factsheets/fs360/en/
   (Date Accessed: 7/9/2015)
2. United Nations and AIDS. Global report on HIV: Annexes; 2012.
   Available:http://www.unaids.org/
   (Date Accessed: 24/7/2015)
3. National Population Census. Federal Republic of Nigeria. Population and housing census. Priority Table 111. Population Distribution by Sex, State, LGA and Senatorial Districts; 2006.
   Available:www.population.gov.ng/files/nationafinal.pdf
   In: Federal Republic of Nigeria. Global AIDS Response Country Progress Report, Nigeria; 2012.

Available:http://ghdx.healthdata.org/record/nigeria-global-aids-responce-country-progress-report-2012
(Date accessed: 24/7/2015)

4. United Nations Development Programme. Human development report on HIV. The rise of the south-human progress in a diverse World; 2013.
Available:http://hdr.undp.org
(Date Accessed: 8/7/2015)
5. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. Journal of American Medical Association. 2003; 289:1008-1011.
6. Akinwale OP, Afilaka B, Gyang VP, Adeleke MA, Adeneye A, Onwujekwe D, Alimi F, Akande DO. Human cytomegalovirus infection in Nigerians living with human immunodeficiency virus. Annals of Tropical Medicine and Public Health. 2013;6:59-64.
7. Springer KL, Weinberg A. Cytomegalovirus infection in the era of HAART: Fewer reactivations and more immunity. Journal of Antimicrobial Chemotherapy. 2004; 54:582–586.
8. Rawlinson W, Scott G. Cytomegalovirus: A common virus causing serious disease. Aust. Fam. Physician. 2003;32:789-793.
9. Feng-Qin F, Qi-Shi F, Zhi-Jun Y, Yi-Bing P, Li Z, Ke-Zi M. Incidence of cytomegalovirus infection in Shanghai, China. Clinical Vaccine Immunology. 2009; 16(11):1700–1703.
10. Drew WL, Sweet ES, Miner RC, Mocarski, ES. Multiple infections by cytomegalovirus in patients with acquired immunodeficiency syndrome: Documentation by Southern blot hybridization. Journal of Infectious Diseases. 1984;150:952-953.
11. Mehrkhani F, Jam S, Sabzvari D, Fattahi F, Kourorian Z, Seyed Alinaghi S. Cytomegalovirus co-infection in patients with human immunodeficiency virus in Iran. Acta Medica Iranica. 2011;49(8):551-555.
12. Kempen JH, Jabs DA, Wilson LA, Dunn JP, West SK, Tonascia J. Mortality risk for patients with cytomegalovirus retinitis and acquired immune deficiency syndrome. Clinical Infectious Diseases. 2003; 37(10):1365-1373.
13. 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):749-751.
14. Sinclair J, Sissons P. Latent and persistent infections of monocytes and macrophages. Intervirology. 1996;39(5-6):293-301.
15. Ojide CK, 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.
16. Adjei AA, Armah HB, Gbagbo F. Seroprevalence of HHV-8, CMV and EBV among the general population in Ghana, West Africa. BMC Infectious Diseases. 2008;8:111-118.
Available:http://dx.doi.org/10.1186/1471-2334-8-111
17. Chakravarti A, Kashyap B, Metlani M. Cytomegalovirus infections: An Indian perspective. Indian Journal of Medical Microbiology. 2009;27:3–11.
18. Guibert G, Warszawski J, Le Chenadec J, Blanche S, Benmebarek Y, Mandelbrot L. Decreased risk of congenital cytomegalovirus infection in children born to HIV-1–infected mothers in the era of highly active antiretroviral therapy. Clinical Infectious Diseases. 2009;48:1516–1525.
19. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. Pediatric Infectious Diseases Journal. 1996; 15:1102–1106.
20. Baig MA, Ali S, Javed R, Khan M, Tabrez S, Subkowitz D, Viera J. Cytomegalovirus gastritis in immunocompetent patient; case report and review of literature. The Internet Journal of Infectious Diseases. 2006;5:1-5.
21. Center for Disease Control and Prevention (CDC). Cytomegalovirus (CMV) and congenital CMV infection; 2010.
Available:http://www.cytomegalovirus
(Retrieved on: 05/06/2011)
22. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, foetus and newborn infant. Clinical Microbiology Reviews. 2002;51(4):680-715.
23. Musa AM, Taura DW, Mukhtar MD, Koki YA, Adamu S. Studies on cytomegalovirus infection among HIV positive patients attending infectious diseases hospital, Kano state. Nigeria. Greener Journal of Epidemiology and Public Health. 2014; 2(1):032-036.

24. Lashini H, Goodarzi Z, Hosseini MJ, Saberfar E. Development of PCR assay for early detection of CMV infection in renal transplant recipients. Journal of Nephro-Urology Monthly. 2011;3(2):106-108.
25. Irna S, Sunardhi W, Tony SDA. Study of cytomegalovirus serology among HIV-infected patients in the highly active antiretroviral therapy era. MKB. 2013; 45(2):112-117.
26. Adeola F, Iheanyi OO, Olajide OA, Suleiman ST. High seropositivity of IgG and IgM antibodies against cytomegalovirus (CMV) among HIV-1 seropositive patients in Ilorin, Nigeria. African Health Science. 2015;15(1):1–9.
27. Ojide CK, Kalu EI, Nwadike VU, Ogbaini-Emovon E, Omoti C. Seroprevalence of cytomegalovirus among HIV-infected adult patients on HAART. International Journal of Tropical Disease and Health. 2013; 3(3):233-241.
28. Ibrahim MK, Ballah AD, Bukar B, Baba GW, Mohammed AT, Ibrahim U, Salisu AK, Ibrahim SK, Haruna Y. Seroprevalence of and risk factors for cytomegalovirus among HIV-infected patients at University of Maiduguri Teaching Hospital, Nigeria. Scientific Research an Academic Publisher. 2014; 4(2):54-59.
29. Akinbami AA, Akanmu AS, Adeyemo TA. Seroprevalence of CMV antibodies among HIV patients and blood donors at Lagos State University Teaching Hospital. South African Medical Journal. 2009;99:528-530.
30. Ledru E, Diagbouga S, Ledru S. A study of toxoplasma and cytomegalovirus serology in tuberculosis and in HIV-infected patients in Burkina Faso. Acta Tropica. 1995;59: 149-154.
Available:http://dx.doi.org/10.1016/0001-706X(95)00073-N
31. Yeroh M, Aminu M, Musa BOP. Seroprevalence of cytomegalovirus infection amongst pregnant women in Kaduna state, Nigeria. African Journal of Clinical Experimental Microbiology. 2015; 16(1):37-44.
32. Manjusha P, Shailja P, Pande RC, Singh KP, Harshita G. Prevalence of cytomegalovirus infection among HIV sero-positive patients – case study from North India. International Journal of Innovation and Scientific Research. 2014;11(1):48-52.
33. Ray K, Mahajan M. Serprevalence of cytomegalovirus antibodies in patients attending STD and antenatal clinics. Journal of Communicable Diseases. 1997; 28(2):85-90.
34. Hizal K, Danks DM, Gibas H, Jack I. Cytomegalovirus in human milk. National England Journal of Medicine. 1972; 287:117–178.
35. Turbadkar D, Mathur M, Dele M. Seroprevalence of TORCH infection in bad obstetric. Indian Journal of Medical Microbiology. 2000;21(2):108–110.
36. Akinbami AA, Akanmu AS, Adeyemo TA, Wright KO, Dada MO, Dosunmi AO. Cytomegalovirus antibodies among immunocompromised (HIV) patients at lagos university teaching hospital (LUTH) Idi Araba, Lagos. Journal of Medicine. 2010;151–154.
37. Chakravarti A, Tewari S, Bhalla P. Human cytomegalovirus infection among patients living with AIDS in a tertiary level hospital in India. Journal of the International Association of Physicians in AIDS Care. 2010;9:94–97.
38. Mujtaba S, Varma S, Sehgal S. Cytomegalovirus co – infection in patients with HIV/AIDS in North India. Indian J. Med. Pes. 2003;117:99–103.
39. Neusa M, Antonio F, Incilia C. Research of antigen and antibodies from retrovirus, CMV, and HBV among prisoners of pententiary complex of the region of comparinces. S.P. Brazilian Review International Medicine Tropical South Paulo. 1998;40(4).
40. Adeola F, Iheanyi OO, Olajide OA, Suleiman ST. High seropositivity of IgG and IgM antibodies against cytomegalovirus (CMV) among HIV-1 seropositive patients in Ilorin, Nigeria. African Health Science. 2015;15(1):1–9.
41. Tsertsvadze T, Gochitashvili N, Sharvadze L, Dvali N. Cytomegalovirus infection in HIV patients. International Conference on AIDS. Infections Disease; AIDS and Clinical Immunology Research Center, Tbilisi, Georgia. 2002;1–2.
42. Neto WC, Rubin R, Shulter J, Giugliana R. Newborn screening for congenital infectious disease. Emerging Infectious Diseases. 2004;1(6):1069-1073.
43. Nishimura N, Kimura H, Rabuta Y, Tanaka N, Ishikawa K, Suzuki C, Morishin T. Prevalence of material cytomegalovirus (CMV) antibody and detection of CMV DNA in amniotic fluid. Medical Immunology. 1999;43(8):781–784.

44. Ludwig A, Hengel I. Epidemiological impact and disease burden of congenital cytomegalovirus infection in Europe. Journal of Europe Surveillance. 2009; 14(9):19140.
45. Okwori A, Olabode A, Emumwen E. Sero-epedemiological survey of cytomegalovirus infection among expectant mothers in Bida, Nigeria. The Internet Journal of Infectious Diseases. 2008;6(2):1-5.
46. Zhong XX, Ma TY. A clinical study of cytomegalovirus infection during pregnancy. Journal Medicine University. 1999;13:60–64.
47. Matos SB, Meyer R, Lima WFM. Seroprevalence of cytomegalovirus infection among healthy blood donors in Bahia State, Brazil. Revista Brasileira de Hematologia eHemoterapia. 2010;3(1): 1516-8484.
48. Ngangom Lilavati, Biswajeet Sahoo, Rebachandra Singh H, Brajachand Singh NG, Sulochana Devi KH, Henkhoneng Mate PH. Co-Infection of cytomegalovirus infection among newly diagnosed HIV infected patients at R.I.M.S. Imphal, Hospital, Manipur. IOSR Journal of Pharmacy and Biological Sciences. 2015; 10(3):06-11.
49. Compston LI, Li C, Sarkodie F, Owusu-Ofori S, Opare-Sem O, Allain JP. Prevalence of persistent and latent viruses in untreated patients infected with HIV-1 from Ghana, West Africa. Journal of Medical Virology. 2009;81:1860–1868.
50. Redwan NA, Ahmed MMM, AL Awfi MS H. Prevalence study of cytomegalovirus (CMV) infection among foreign manpower in Jeddah Saudi Arabia. African Journal of Microbiology Research. 2011;5(17):2539-2549.
51. Maingi Z, Nyamache AK. Seroprevalence of cytomegalo virus (CMV) among pregnant women in Thika, Kenya. BioMed Central Research Notes. 2014;7:794.
52. Umeh EU, Onoja TO, Aguoru CU, Umeh JC. Seroprevalence of cytomegalovirus antibodies in pregnant women, Benue State, Nigeria. Journal of Infectious Diseases and Therapy. 2015;3:242.
DOI: 10.4172/2332-0877.1000242

APPENDIX I

Ethical approval for the conduct of the research study

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

23rd Dec., 2014

Aliyu, A

Ethical approval

Sequel to the submission of the research proposal to the Advisory Committee on research of the university on a research study titled: **Seroprevalence And Molecular Detection Of Human Cytomegalovirus In HIV Positive Patients Attending Some Selected Hospitals In Kaduna State, Nigeria.** The committee after a long considerations and consultations has satisfied with the research.

The ethical committee thereby grants ethical approval for the study.

Dr. Ado, AS

Head of Department Microbiology,
For: Dean, Postgraduate Studies
Ahmadu Bello University, Zaria, Nigeria.

APPENDIX II

Sample of the patients' consent form

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

Serial No.: ..... *Hospital No.:* ..... *Age:* ..... *Phone No.:* .....

This Informed Consent Form is basically for immunocompromised (HIV) patients attending some selected Hospitals in the three senatorial district of Kaduna State. We are inviting you to participate in this research work titled "***"Seroprevalence and Molecular detection of Human Cytomegalovirus in immunocompromised (HIV) patients in Kaduna State Nigeria"***.The presentation of Human Cytomegalovirus disease ranges from subclinical infection which may lead to hearing, cognitive and motor impairment. Clinical syndromes that may be observed include; encephalitis, pneumonitis, hepatitis, retinitis, colitis and grafi rejection, microcephaly, mental retardation, spastic paralysis, hepatosplenomegaly, anemia, thrombocytopenia, deafness and optic nerve atrophy leading to blindness. Differences in virus strains, as well as incompletely understood host immune factors are likely responsible for the range of clinical symptoms. The research will involve the collection of blood samples of HIV patients. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I .....of ..... do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that blood sample will be taken for the test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant .....
Signature/right thumb print of participant ..... Date .....

Statement by Witness:
I have witnessed the accurate reading of the consent form and detail explanation of the study to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness.....
Signature of witness..... Date .....

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant will be given an opportunity to ask questions about the study, and all the questions asked by participant will be answer correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher .....

Signature ..... Date. .....

APPENDIX III

Sample of the research study Questionnaire

RESEARCH QUESTIONNAIRE

We the researchers of Microbiology Department, Faculty of Science Ahmadu Bello University, Zaria wish to seek for your consent in taking sample of HIV patients in order to carry out our research study on the topic **SEROPREVALENCE AND MOLECULAR DETECTION OF HUMAN CYTOMEGALOVIRUS IN HIV PATIENTS ATTENDING SOME SELECTED HOSPITALS IN KADUNA STATE**, which involves collection of blood sample. Kindly assist by ticking the appropriate information which will be treated and kept confidential.

SECTION A: BIODATA

1. Reference no: ……..
2. Occupation: ……
3. Age: ……
4. Sex: Male [ ] Female [ ].
5. Marital Status: Single [ ] Married [ ] Divorced [ ] Widowed [ ] Widower [ ].
6. Place of resident: Kaduna North [ ] Kaduna Central [ ] Kaduna South [ ] Others, please specify……

SECTION B: DEMOGRAPHIC DATA AND RISK FACTORS FOR CYTOMEGALOVIRUS

7. Level of education: Primary [ ] Secondary [ ] Tertiary [ ] Quranic [ ] Not educated [ ]
8. Type of marriage: Monogamy [ ] Polygamy [ ] Not Available [ ]
9. Number of people in the household .....
10. Lifetime history of multiple (more than one) sexual partners. Yes [ ] No [ ]
11. Preferred mode of sexual activity Homo [ ] Hetero [ ]
12. Frequent practices of undergoing through tribal marks and traditional acupunture Yes [ ] No [ ]
13. Taking pareteral hard drugs Yes [ ] No [ ]
14. Have you been transfused with blood? Yes [ ] No [ ]
15. Is there history of temporary deafness or partial blindness? Yes [ ] No [ ]
16. Are you on Antiretroviral Therapy: Yes [ ] No [ ]
17. For how long……..

SECTION C: CLINICAL INFORMATION

What are the symptoms of the ailment?

18. Any miscarriages/abortion? Yes [ ] No [ ] NA [ ]
19. Fever: Yes [ ] No [ ]
20. Fatigue: Yes [ ] No [ ]
21. Headache: Yes [ ] No [ ]
22. Low breathing: Yes [ ] No [ ]
23. Nose bleeding: Yes [ ] No [ ]

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