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# CYTOMEGALOVIRUS IGM ANTIBODY DETECTION AND HEPATITIS B AND C COINFECTIONS AMONG HIV PATIENTS IN ADO-EKITI, NIGERIA

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**ABSTRACT:** *Opportunistic viral infections such as Cytomegalovirus (CMV), Hepatitis B (HBV) and C (HCV) are often prevalent in HIV patients undergoing antiretroviral therapy (ART) thereby shortening the life span of infected patients. The co-infection of CMV, HBV and HCV among HIV positive patients attending ART clinics across hospitals in Ekiti State, Nigeria, were studied. Ninety-two (92) blood samples from HIV positive individuals (males, pregnant females and non-pregnant females) were collected and serologically screened for CMV antibodies (IgM) using enzyme linked immunosorbent assay (ELISA) while HBV and HCV surface antigen were determined using standard test kit. The viral loads were determined using COBAS® AmpliPrep / COBAS® TaqMan® systems. The occurrence of single or coinfection of HIV-patients with respect to their sampling class were also determined and expressed in percentage. Of samples screened, the total positive occurrence rates of 23.16±1.78%, 11.20±1.46% and 30.21±2.67% were observed for CMV, HBV and HCV while 35.43±1.85% of total respondents tested negative to all the viruses screened in this study. The total viral loads were 10184 copies/ml for CMV, 5679 copies/ml for HBV and 12678 copies/ml for HCV. For the coinfections, respondents with HBV+HCV had a total occurrence of 31.80±2.25% (13294 copies/ml) while HCV+CMV had total occurrence of 44.40±3.18% (51783 copies/ml), CMV+HBV had total occurrence of 19.25±1.67% (8679 copies/ml) and CMV+HCV+HBV had a total occurrence of 9.50±1.35% (586 copies/ml). This high prevalence rate of CMV with Hepatitis co-infection among these HIV positive individuals further support the role of viral reactivation in immunocompromised patients.*

**KEYWORDS:** Co-infection, HIV, CMV, HBV, HCV, ELISA, qPCR

## INTRODUCTION

The most common Opportunistic and coinfections in HIV infected individuals reported are tuberculosis, chronic diarrhoea, oral candidiasis, herpes simplex virus-2, cytomegalovirus, hepatitis B virus and hepatitis C virus (Kallol *et al*., 2011). Following the emergence of Acquired Immunodeficiency Syndrome (AIDS), opportunistic infections caused by various biological agents became one of the main public health concerns relative to individuals living with the immunodeficiency virus HIV/AIDS (Silva, *et al*., 2015). Antiretroviral therapy (ART) is able to restore the patients' immunity, increasing the number of CD4+ T lymphocytes to their normal values, and it is also able to reduce the HIV viral load (Brantsaeter *et al*,. 2007). In spite of the therapeutic and preventive measures available for the control of infection, epidemiological data indicate that a significant number of individuals are still infected by HIV, particularly youths. Cytomegalovirus (CMV) stands out among the main causes of secondary infections to AIDS

because it seemingly acts as an inducer or co-factor in the progression of HIV infection pathogenesis (Silva *et al*., 2015).

HIV-infected individuals are almost universally co-infected with CMV (Gianella *et al*., 2015), and both viral infections are associated with inflammation and aging (Effros, 2015). The CMV seems to exert a more dramatic effect than HIV (in HIV RNA–suppressed individuals) and might be the so-called "smoking gun" of immune-senescence among co-infected persons (Pathai *et al,* 2014). The main differential effect between CMV and HIV seems to be their impact on $CD8^+$ T cells (Klenerman and Oxenius, 2016). A recent study found elevated numbers of $CD8^+$ T cells and a low $CD4^+/CD8^+$ T-cell ratio in individuals co-infected with both viruses but not in persons infected with HIV alone or CMV alone (Freeman *et al.,* 2015). Along the same line, HIV-infected individuals who are seronegative for CMV show greater resilience and better immune recovery following ART (Barrett *et al.,* 2014). The incidence of life-threatening complications has decreased dramatically with suppressive combination ART, likely due to restoration of CMV-specific immune responses that limit CMV reactivation (Deayton *et al*., 1999). While the clinical importance of CMV in the setting of ART-treated HIV disease is less clear, emerging evidence links CMV to suboptimal immune response to ART (Barrett *et al.*, 2014) and increased risk of non–AIDS-related complications (Lichtner *et al*., 2015). The event most frequently associated with CMV was cardiovascular disease, which has been described following organ transplantation (Watkins *et al*., 2012) and with HIV disease (Parrinello *et al*., 2012).

Majority of the persons infected with HIV also may occasionally develop severe hepatobiliary problems pertaining to which liver complications like hepatitis B and hepatitis C are on the rise (Saravanan *et al,* 2007). It is reported that among the total cases of HIV infections worldwide, 2-4 million are estimated to have chronic hepatitis B Virus (HBV) co-infection, while 4-5 million are co-infected with hepatitis C virus (HCV) (Alter, 2006). Chronic infections with viruses such as cytomegalovirus (CMV), hepatitis B virus (HBV), and hepatitis C virus (HCV), which are more prevalent in HIV-infected populations, have been linked to immune dysfunction and decreased vaccine response in the general population (Troy *et al.,* 2016).

Udeze *et al*. (2018), Kolawole *et al*. (2016), Pateker *et al*. (2015) and Silva *et al*. (2015) among others, have investigated viral co-infection among HIV subjects, however, there are not enough information on multiple viral co-infection such as Hepatitis and CMV among HIV positive individuals. Therefore, this study evaluated the co-infection in HIV patients attending a Government hospital with respect to CMV, HBV and HCV infections.

## METHODS

### Study design and population

This was a cross-sectional, hospitals-based study. The study included 92 participants (14 males, 30 pregnant females and 48 non-pregnant females; age range 18– 61 years), confirmed HIV-infected individuals attending an anti-retroviral treatment centers across the senatorial districts in Ekiti state.

**Sample collection and preparation**

About 5ml of blood sample was aseptically obtained from each participant into a sterile bottle and allowed to clot at room temperature, then centrifuged at 3000rpm for 10 minutes. Then serum was aspirated into new transferred into cryovials appropriately labelled and stored at −20°C until assayed.

**Serology**

Serum samples were tested for the presence of IgM available Enzyme Linked Immunosorbent Assay (ELISA) kit (manufactured by Melsin Medical Co., Limited. China) for detection of CMV specific IgM. The tests were performed and interpreted according to the kits' manufacturer's instructions while HBV and HCV surface antigen were determined using standard test kit. The viral loads were determined using the COBAS® AmpliPrep / COBAS® TaqMan® HIV-1 Test, v2.0, which is based on isolating the HIV-1 RNA, automated reverse transcription of the target RNA to generate complementary DNA (cDNA), PCR amplification, detection of HIV-1 target RNA and HIV-1 quantification standard (QS) Armored RNA. This analyzer calculates the HIV-1 RNA concentration in the test specimens by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control.

**Ethical clearance**

Ethical clearance was obtained from the Ethical Review Committee (ERC) of Hospital Management board Ekiti State, Nigeria (Ref No: HMB/AD.567$^{T}$/3).

**Statistical analysis**

Data was analyzed using GraphPad Prism 5.0., Pearson Moment Correlation of the HBV, HCV and CMV while Descriptive statistics of Frequency count, percentages and Bar graphs were also used for better explanation. A p value of $<0.05$ was considered significant.

## RESULTS

The occurrence of single viral infections such as HCV, HBV and CMV alone are represented in Table 1 and analyzed across the sampling classes (males, pregnant females and non-pregnant females) as percentages in Figure 1. The occurrence of the HCV alone was 35.71±2.55% for males, 20.50±1.75% for pregnant females and 35.42±2.82% for non-pregnant females while occurrence for HBV alone was 14.29±1.86% for males, 6.67±1.94% for pregnant females and 14.58% for non-pregnant females. The occurrences for CMV also stood at 21.43±2.78% for males, 13.33±1.67% for pregnant females and 29.17±2.18% for non-pregnant females. The viral loads for each single infection (CMV, HBV and HCV) are represented in Figure 2. The viral load for CMV in males was 1454.85 copies/ml, 1939.81 copies/ml for pregnant females and 6789.33 copies/ml for non-pregnant females. The viral load for HCV in males was 2263.93 copies/ml, 2716.71 copies/ml in pregnant females and 7697.36 copies/ml in non-pregnant females. The viral load for HBV was 1032.55 copies/ml in males and 1032.55 copies/ml in pregnant females while it was 3613.91 copies/ml in non-pregnant females.

Table 1: Percentage occurrence of viral infection in HIV-patients

| Viruses | Seroprevalence | Viral infection (%) |
|---|---|---|
| CMV | 21 | 23.16±1.78% |
| HBV | 11 | 11.20±1.46% |
| HCV | 28 | 30.21±2.67% |
| HIV only | 32 | 35.43±1.85% |
| Total | 92 | 100 |

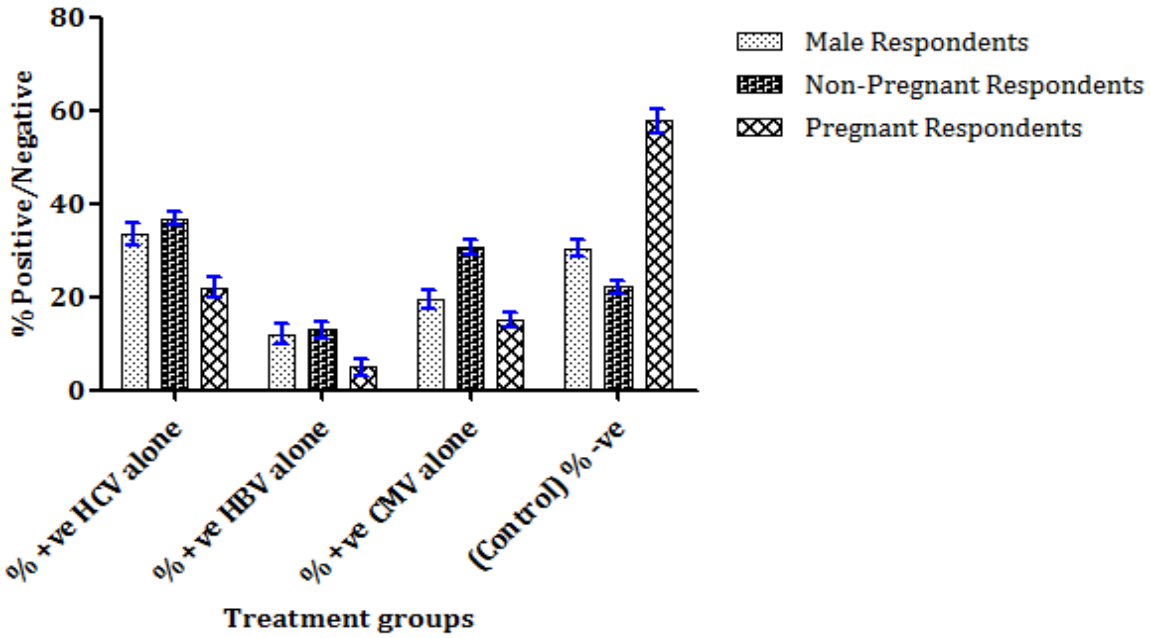


Figure 1: Percentage occurrence for single viral infections in HIV-patients

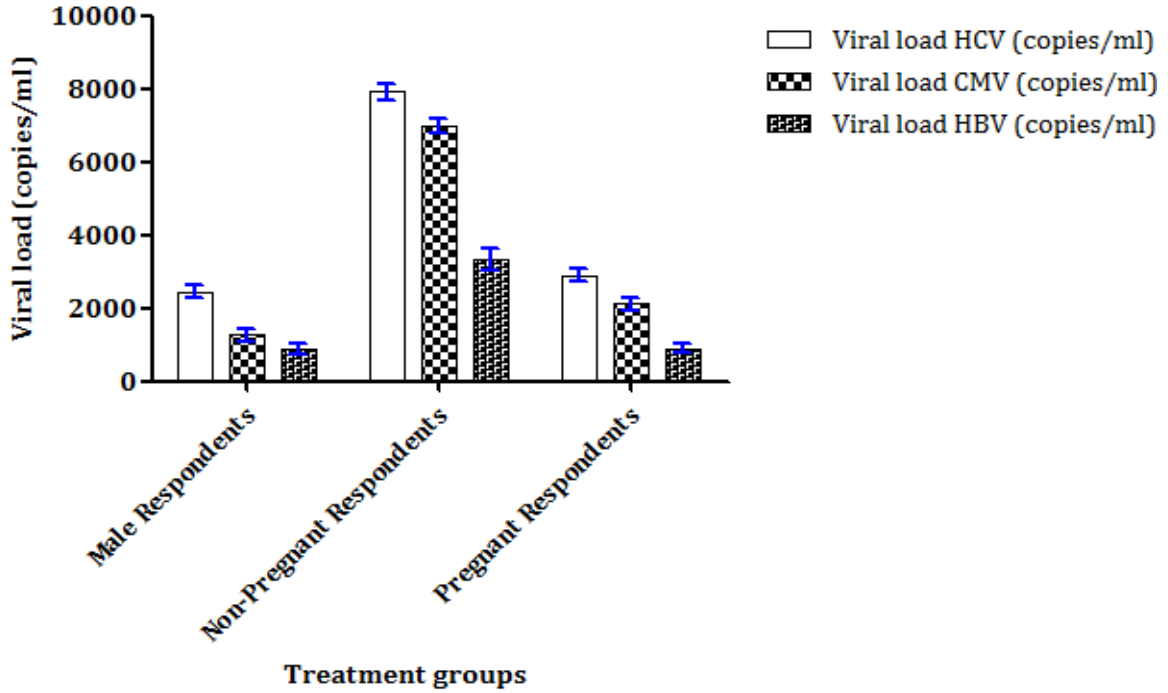


Figure 2: Viral load of single infections in HIV-patients

Conversely, the occurrence of the viral co-infections such as HCV+HBV, HBV+CMV, CMV+HCV and CMV+HCV+HBV are represented across the sampling classes (males, pregnant females and non-pregnant females) as percentages in Figure 3. The occurrence of the HCV+HBV was 28.75±1.69% for males, 10.19±1.21% for pregnant females and 45.82±2.59% for non-pregnant females while occurrence for HBV+CMV was 18.29±1.86% for males, 29.67±1.94% for non-pregnant females and none for pregnant females. The occurrence of CMV+HCV also stood at 50.45±2.25% for males, 16.67±1.33% for pregnant females and 58.33±2.77% for non-pregnant females. Occurrence of HCV+CMV+HBV stood at 14.29±1.88% in males, none in pregnant females and 12.50±1.50% in non-pregnant females. The viral loads for co-infection cases (HCV+HBV, HBV+CMV, CMV+HCV and CMV+HCV+HBV) are represented in Figure 4. The viral load for HCV+HBV in males was 1833.66 copies/ml, 1375.24 copies/ml in pregnant females and 10085.10 copies/ml for non-pregnant females. The viral load for HBV+CMV in males was 1446.50 copies/ml, none in pregnant females and 7232.50 copies/ml in non-pregnant females. The viral load for CMV+HCV was 9062.03 copies/ml in males and 6472.88 copies/ml in pregnant females while it was 36248.10 copies/ml in non-pregnant females. The viral load of CMV+HCV+HBV in males was 146.50 copies/ml, none in pregnant females and 439.5 copies/ml in non-pregnant females respectively.

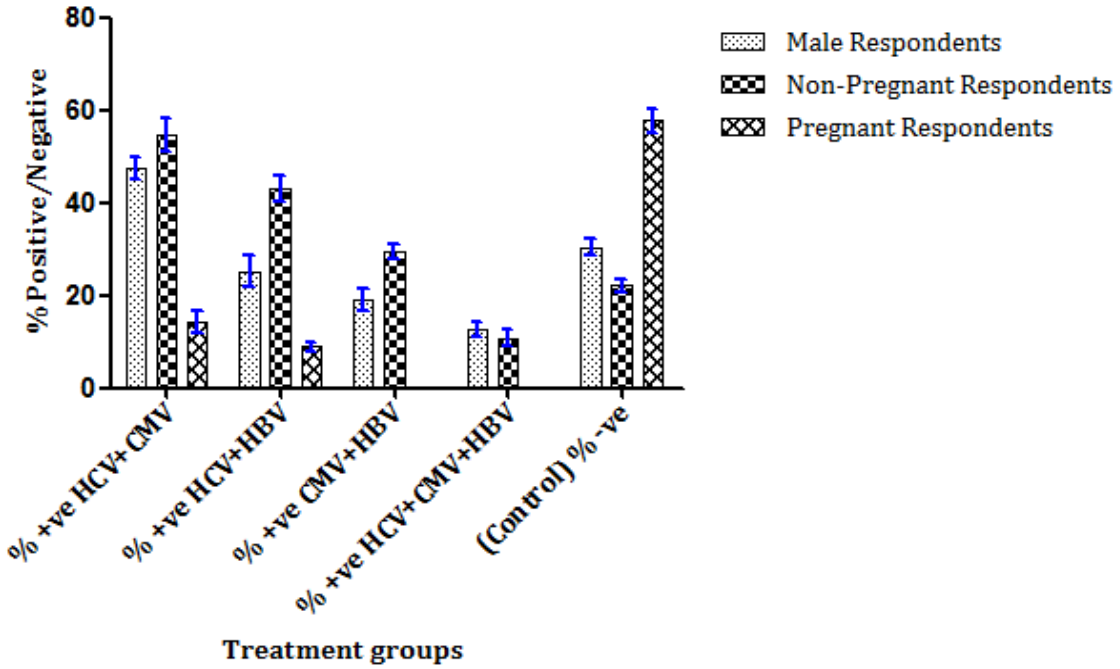


Figure 3: Percentage occurrence for viral co-infections in HIV-patients

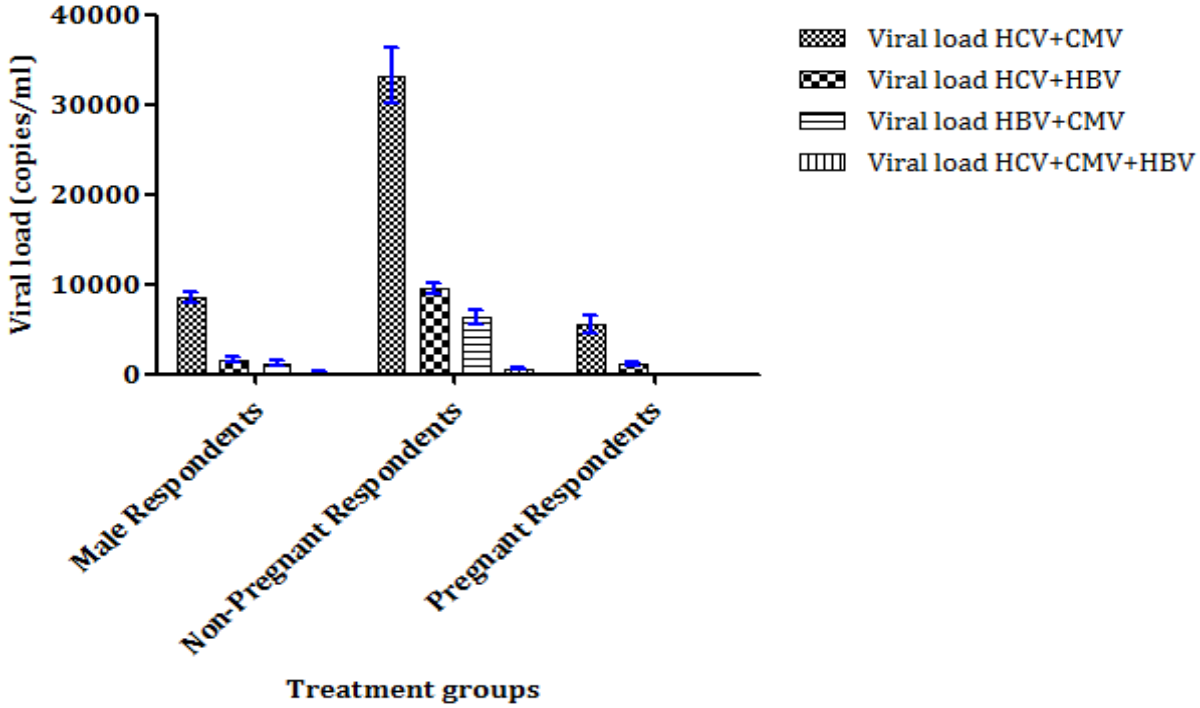


Figure 4: Viral load of co-infections in HIV-patients

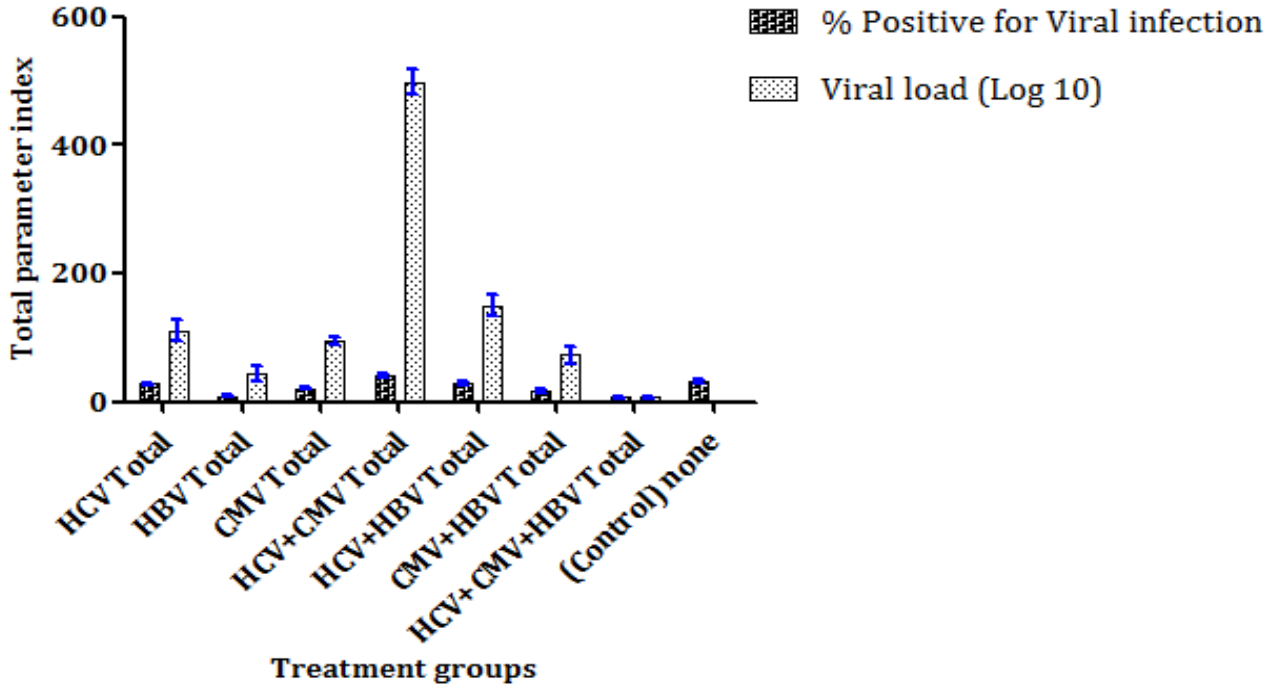


Figure 5: Total occurrence and viral loads of the single and co-infections in HIV patients

Conversely, the Figure 5 separately shows the total occurrence rates in percentages as well as the total viral loads of all the single (CMV, HCV, HBV) and the co-infections (HCV+HBV, HBV+CMV, CMV+HCV and CMV+HCV+HBV) obtained in this study.

## DISCUSSION

Chronic infections with viruses such as cytomegalovirus (CMV), hepatitis B virus (HBV), and hepatitis C virus (HCV), which are more prevalent in HIV-infected populations, have been linked to immune dysfunction and decreased vaccine response in the general population (Ferrari, 2015). Given similar behavioral risk factors for acquisition, HIV-infected adults generally have high rates of CMV, HBV, and HCV co-infection (Troy *et al.*, 2016). The CMV infection has been hypothesized to be associated with immune-senescence, or immune aging, and chronic immune activation (Wittkop *et al*., 2013). Chronic HBV and HCV infection have each been associated with immune exhaustion and increased expression of inhibitory lymphocyte receptors (Yao and Moorman, 2013; Ye *et al.*, 2015). Even with the widespread use of effective combination antiretroviral therapy (cART), HIV-infected adults still have a 35-fold higher rate of invasive pneumococcal disease, a 73-fold higher rate of influenza-related mortality, a 10-fold higher rate of invasive meningococcal disease, and a 19-fold higher rate of chronic hepatitis B virus (HBV) infection (Laurence, 2005; Udeze *et al*., 2018).

The occurrence rates of CMV, HBV and HCV respectively which is relatively higher than the other study groups could be as a result of poor hygiene, low educational status and are mostly small scale business owners that pre-dispose them to the virus. The age bracket 21-40 years

represent the sexually mature and active adults with the tendency towards sexual promiscuity and are therefore most likely to experience higher infection (Esumeh *et al*., 2003). The result obtained also underscore why the risk of single and multiple viral co-infections is significantly high and indicates the level of on-going and overall burden of the infection among the HIV infected patients in Ekiti state. However, the occurrence of CMV is higher than earlier studies by Udeze *et al.* (2018) and Fowotade *et al*. (2015); this indicate significant level of chronic ongoing CMV infection HIV infected individuals in the area of research study. In comparison with earlier study of Otegbayo *et al.* (2008), the total occurrence of HBV in this study (11.20±1.46%) is not statistically different from the occurrence recorded in his study (11.9%), while Adoga *et al.* (2009) reported HBV at 6.6% and Adekunle *et al.* (2011) reported HBV at 2.7%. Meanwhile, this study reported HCV at 30.21±2.67% significantly higher than 15.5% reported in Forbi *et al.* (2007), 25.9% reported in Uneke *et al*. (2005), and 28.4% documented in Balogun *et al*. (2012). Figures for HCV as high as 51.9% and 70.5% have equally been documented in Iwalokun *et al.* (2006) and Nwokedi *et al.* (2006). This high HCV load further justifies its high efficiency of transmission via blood contact and mother to child. Conversely, total co-infection occurrences reported in this study were generally higher than 7.0% reported in Ojide *et al*. (2015), 8.2% reported in Agwale *et al*. (2004), 14.7% reported in Balogun *et al*. (2012) and 6.5% reported in Bùi *et al.* (2014). Furthermore, the percentage of respondents who tested negative to all the single and co-infection cases in this study (35.43±1.85%) was significantly higher than 10.5% documented in Deborah *et al.* (2015) and 16.98% reported in Balogun *et al*. (2012).

## CONCLUSION

The high prevalence rate of CMV with Hepatitis co-infection among these HIV positive individuals pose a threat in our environment and further support the role of viral reactivation in immunocompromised patients. Further research is necessary to investigate blood or serum markers that could arise due to this co-infections which will serve as a guide towards control measure.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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