High Burden of On-Going HSV-1 and -2 Infections in Human Immunodeficiency Virus-Infected Individuals in a Secondary Healthcare Facility in Imo State, Nigeria

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Abstract: Because of shared route of transmission of human immunodeficiency virus type 1 (HIV-1), herpes simplex virus type 1(HSV-I) and type 2 (HSV-2), HIV-1-infected persons are also susceptible to infection by HSV-1 and HSV-2. This study aimed at determining the prevalence of HSV-1 and HSV-2 IgM antibodies among HIV-infected individuals accessing healthcare at General Hospital, Awo-Omamma Imo state, Nigeria. This is a hospital-based cross-sectional study. Blood samples were collected from 182 (38 males and 144 females: age range 3-72 years; mean age 36.5 years) HIV-infected participants on ART after collecting pertinent socio-demographic data using questionnaires. Serum from each sample was tested for the presence of IgM antibodies against HSV-1 and -2 using ELISA. Data were analysed using Chi-squared test and binary logistic regression analysis with SPSS 15.0 for Windows. Of the 182 samples tested, 166 (91.2%) and 156 (85.7%) were respectively positive for HSV-1 and HSV-2 while 148 (81.3%) were positive for both HSV-1 and HSV-2 IgM antibodies. These respectively represented dual HIV/HSV-1, HIV/HSV-2 and triple HIV/HSV-1/HSV-2 infection rates. Variables analysed as risk factors include patients' gender, age group, marital status, educational status and $CD4^+$ cell count. Age was predictive of HIV/HSV-1 dual infection while gender and age were both predictive of HIV/HSV-2 dual infection rates among the participants. This study reports high seroprevalence of both HSV-1 and HSV-2 IgM antibodies among this cohort. Mass education targeted especially at the most vulnerable groups on the dangers and ways of preventing these infections is recommended.
Keywords: HIV-patients, HSV-1, HSV-2, IgM antibodies, risk factors

INTRODUCTION

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are among the most common co-infections often seen among HIV-infected individuals presumably due to their shared route of transmission. While HIV and HSV-2 are principally transmitted sexually, HSV-1 transmission most frequently occurs via nonsexual routes (direct person to person contact) at childhood. However, increase in the practice of oral-genital sexual practice has led to increase in oral infection with HSV-2 and genital infection with HSV-1. Globally, an estimated 90% to 100% and 52% to 95% of HIV-1 infected individuals are co-infected with HSV-1 and HSV-2 respectively (McFarland *et al*., 1999; van Benthem *et al*., 2001; Suligoi *et al*., 2002; Lama *et al*., 2006; Kapiga *et al*., 2007).

HSVs are known to cause a wide spectrum of clinical manifestations in humans such as central nervous system (CNS), genital and dermal diseases.

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In HIV-infected individuals however, the synergistic effects of the co-infections with these viruses on each other have been well documented (Andreoletti *et al*., 2005; Barnabas *et al*., 2012; Tan *et al*., 2012). Infection with HSV-2 has been shown to affect the acquisition and subsequent course of HIV-1 in several ways. The physical disruption of the epithelial barrier of genital mucosal surfaces caused by HSV-2 infection increases susceptibility to HIV infection by providing increased portals of entry for HIV into the HSV-2-infected person during unprotected sexual contact (Tan *et al*., 2009). Also, the reactivation of the virus at mucosal surfaces as well as asymptomatic HSV shedding leads to recruitment and persistence of inflammatory cells in the genital tract (Wald and Link, 2002; Freeman *et al*., 2006; Ward and Ronn, 2010) and also increases the concentration of HIV-1 in plasma and genital secretions (Schacker *et al*., 2002; Celum *et al*., 2005; Barnabas and Celum, 2012). This leads to increased transmissibility of HIV from persons co-infected with HSV-2 and HIV than from HIV mono-infected persons. In those chronically infected by the virus, approximately 3-fold increase in HIV acquisition rate has been reported (Freeman *et al*., 2006; Johnston *et al*., 2011) and up to 6-fold for high-risk group

like female commercial sex workers (Kaul *et al*., 2004). On the other hand, HSV-1 has been linked with severe morbidity and mortality both in the setting of advanced HIV disease and immunocompetent hosts (Moulignier *et al*., 1996). Studies have shown more frequent oral and genital shedding of both HSV-1 and -2 among those who are co-infected with HIV-1 than among HSV-infected or HIV-1 monoinfections (Augenbraun *et al*., 1995; Schacker *et al*., 1998; Kim *et al*., 2006).

A number of factors are believed to stimulate reactivation and replication of HSV; these include fever, exposure to sun, concurrent viral infection, hormonal changes, stress and possibly localized trauma (Erlich *et al*., 1995). In sub-Saharan Africa where the greatest burden of HIV abounds, it is believed that more than 60% of the general population is infected with HSV-2 (Corey *et al*., 2004). Considerably higher percentage is expected among HIV-infected individuals and an estimated half of the cases can be directly attributed to infection with HSV-2 (Wald, 2004).

In Nigeria, there is a dearth of information on the burden of these co-infections with the few available ones focusing on HIV/HSV-2 co-infection (Mawak *et al*., 2012) with virtually none for co-infection of HIV and HSV-1. This study was therefore conducted to determine the proportion of HIV-1 patients on ARV drugs in Imo state, Nigeria with on-going HSV-1/2 infections and to report associated risk factors with the view to highlighting the need to prevent/control HSVs among HIV-1 patients on antiretroviral therapy (ART).

MATERIALS AND METHODS

Study location and participants

This study was conducted between February and May, 2012 at the General Hospital, Awo-Omamma, Imo state, south-eastern Nigeria. The hospital serves mainly patients from all the 5 states in the region. The study participants included 182 (38 males and 144 females: age range 3-72 years; mean age 36.5 years) HIV-infected individuals accessing antiretroviral treatment in the hospital. They comprised apparently healthy individuals with none on anti-HSV drug during sampling. Sample size was estimated by using established formula ($n = [Z^2PQ] \div d^2$) (WHO, 2004; Niang *et al*., 2006) and a sample size of 304.9 persons was arrived at using 27.3% prevalence of HSV antibody previously reported in Anambra State, South East, Nigeria (Duru *et al*., 2014). However, a sample size of 182 HIV-infected was studied because, among other reasons, sampling 305 HIV-infected persons was logistically prohibitive. In addition, being a ubiquitous viral pathogen with high prevalence rate, 182 persons are enough to detect HSV antibodies if they were ever exposed to the viruses.

Study design

This is a cross-sectional, hospital-based serosurvey. Approval to carry out the study was obtained from Imo State Hospital Management Board with reference number HMB/AD/872/T/2. Each participant provided consent before sampling. Socio-demographic data were collected using interviewer-administered questionnaire. For the purpose of analysis, the participants were divided into two categories based on $CD4^+$ cell count (i.e. ≤ 200 and > 200 $CD4^+$ count categories).

Sampling and blood sample preparation

The consenting HIV-1 infected participants were consecutively selected in the hospital irrespective of age, state of health and gender; exclusion criteria were no willingness to participate and not being on ART. Blood samples were aseptically collected by venepuncture from the HIV-1 infected participants. About 5ml of blood sample was obtained from each participant into sterile bottle and allowed to clot at room temperature before spinning at 3,000 rpm for 10 minutes. Then serum was aspirated into new Eppendorf tubes, appropriately labelled and stored at -20^{o}C until assayed.

Serology

Serum samples were tested for the presence of IgM antibodies using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit (manufactured by Diagnostic Automation, Inc., Calabasas, CA, USA) for detection of HSV-1 and HSV-2 specific IgM antibodies. The tests were performed and interpreted according to the manufacturer's instructions.

Data analysis

Results are presented with descriptive statistics at 95% confidence interval where appropriate. Inferential statistical analyses were done with CHI^2 test and binary logistic regression. The analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL), at $p \leq 0.05$ level of significance.

RESULTS

Of the 182 participants, HSV-1 IgM positivity was found in 166 giving a prevalence rate of 91.2% [95% CI: 87.1- 95.3%]); and HSV-2 IgM positivity of 85.7% (95% CI: 80.6-90.8%), Table 1. These seroprevalence rates were statistically comparable (p=0.10). We observed that 148 of the 182 study participants were positive for both HSV-1 and HSV-2 IgM, thus giving HIV/HSV-1/HSV-2 triple infection rate of 81.3% (95% CI: 75.7-87.0%). Eighteen (9.9% [95% CI: 5.6-14.2%]) were positive for HSV-1 IgM only; 8 (4.4% [95% CI: 1.4-7.4%]) were positive for HSV-2 IgM only while the remaining 8 (4.4% [95% CI: 1.4-7.4%]) had no detectable IgM against either HSV-1 or HSV-2 (Figure 1).

Findings revealed that 94.7% (95% CI: 87.6-101.8%) and 90.3% (95% CI: 85.4-95.1%) of the HIV-infected males and females respectively were positive for HSV-1 IgM antibody. These rates were statistically comparable (p=0.39). However, the females had significantly higher (p=0.02) HSV-2 IgM prevalence rate than the males (females: 88.9% [95% CI: 83.8-94.0%] versus males: 73.7% [95% CI: 64.2-91.4%]) (Table 1). The statistical association of female gender with HSV-2 IgM prevalence reflected as odds ratio (OR) of 2.9 (95% CI: 1.2-6.9). We also observed higher prevalence rates of both HSV-1 and HSV-2 IgM antibodies among the single participants compared to the married (Figure 2).

Table 1: Gender-distribution of HSV-1 and -2 IgM antibodies among the study participants

Gender	No. tested	HSV-1	HSV-2
		No. (%) positive	No. (%) positive
Male	38	36 (94.7)	28 (73.7)
Female	144	130 (90.3)	128 (88.9)
Total	182	166 (91.2)	156 (85.7)

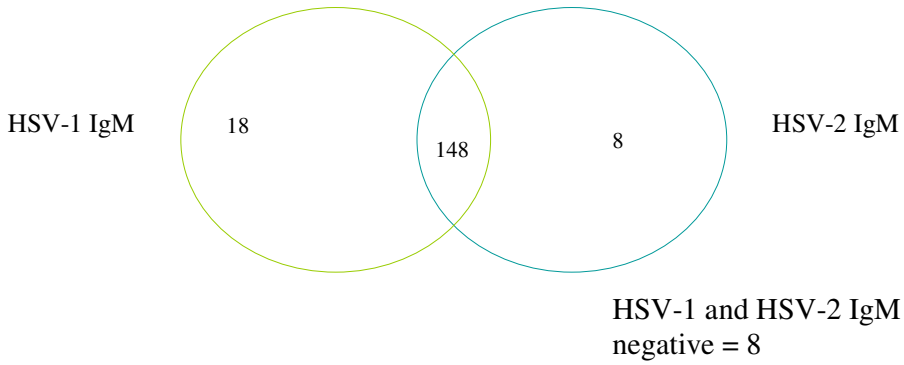


Figure 1: Venn diagram of relationship between HSV-1 and HSV-2 IgM positivity among the HIV-infected study participants

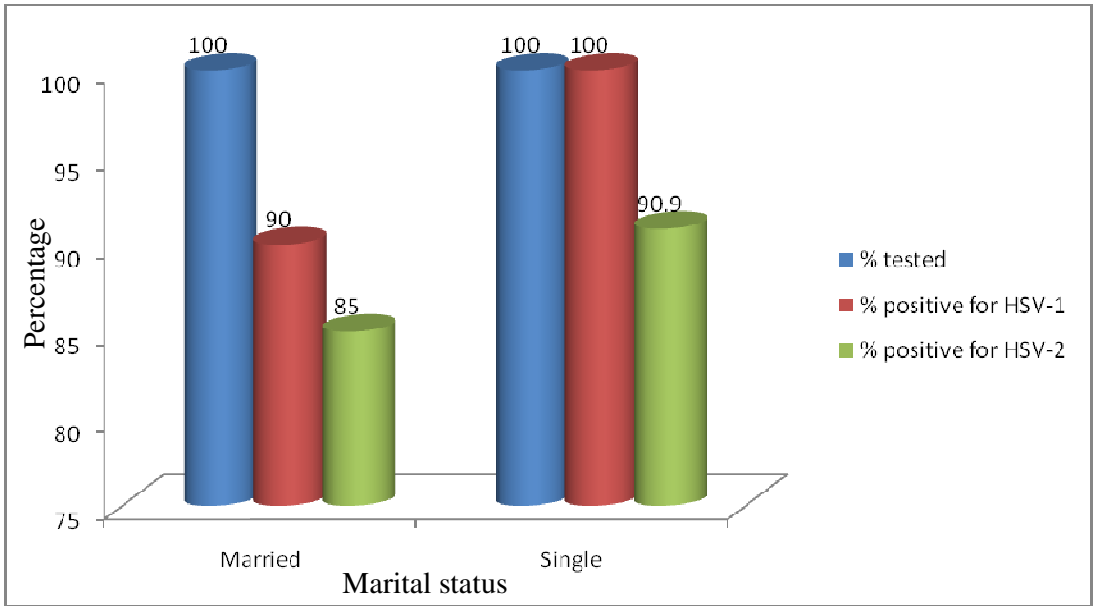


Figure 2: Prevalence rates of HSV-1 and HSV-2 IgM antibodies in both married and single study participants

Regarding age of the participants, the trends of IgM prevalence rates for HSV-1 and -2 IgM are shown in Figure 3. The sample sizes of the age groups were: ≤ 20 yrs (n=4); 21-30 yrs (n=56); 31-40 yrs (n=58); 41-50 yrs (n=42); ≥ 51 yrs (n=14) with those whose ages were unknown (n=8). Participants with age group 21-30 years (n=54) however had significantly higher (p=0.03) prevalence than those in the 31-40 yrs age group, the OR showed that the HIV-1 participants in the 21-30 yrs age group were about 6 times (OR = 5.6) more likely to be HSV-1 IgM positive compared to those in the 31-40 yrs age group. The HIV-1 infected participants in the age groups 21-30 yrs, 31-40 yrs and 41-50 yrs each had independent association (p=0.001 [OR=21.7]; 0.004 [OR=10.4] and 0.02 [OR=7.1] respectively) with HSV-2 IgM prevalence compared those with unknown ages that had the lowest HSV-2 IgM prevalence.

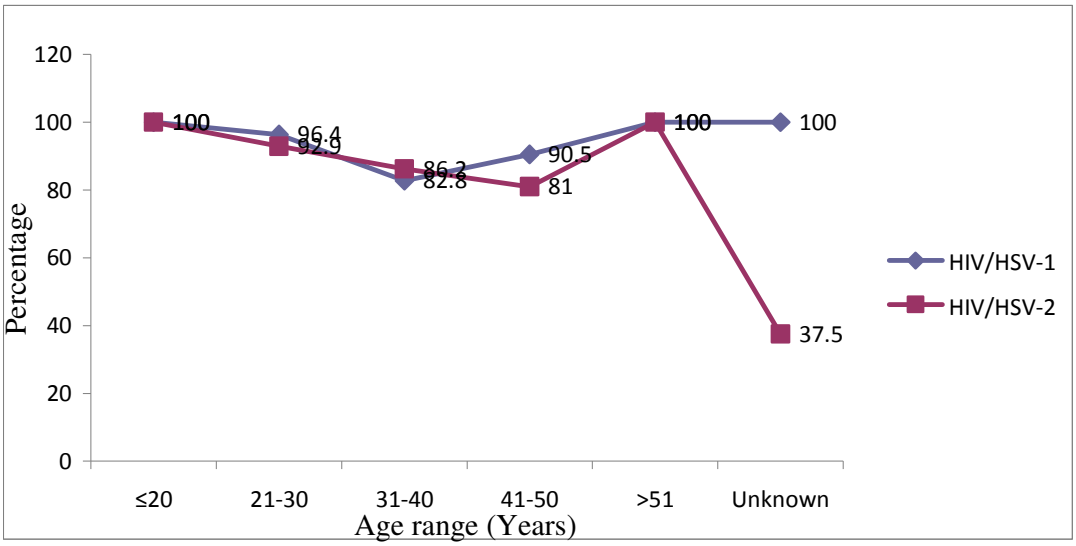


Figure 3: Prevalence rates of HSV-1 and HSV-2 IgM antibodies with respect to age of participants

Figure 4 shows the trends of prevalence rates of HSV-1 and HSV-2 IgM antibodies in relation to educational status of the study participants. The sample sizes for the educational categories are: primary education (n=28); secondary (n=104); tertiary (n=20); no formal education (n=6) and those with unknown educational status (n=24). Binary logistic regression analysis showed that those without formal education were statistically comparable (p=0.9, 0.13, 0.1 and 0.2) to each of the remaining educational groups. As for HSV-1 IgM, the prevalence of HSV-2 IgM among those without formal education was comparable (p=0.13, 0.2, 0.3 and 0.3) to each of the remaining groups.

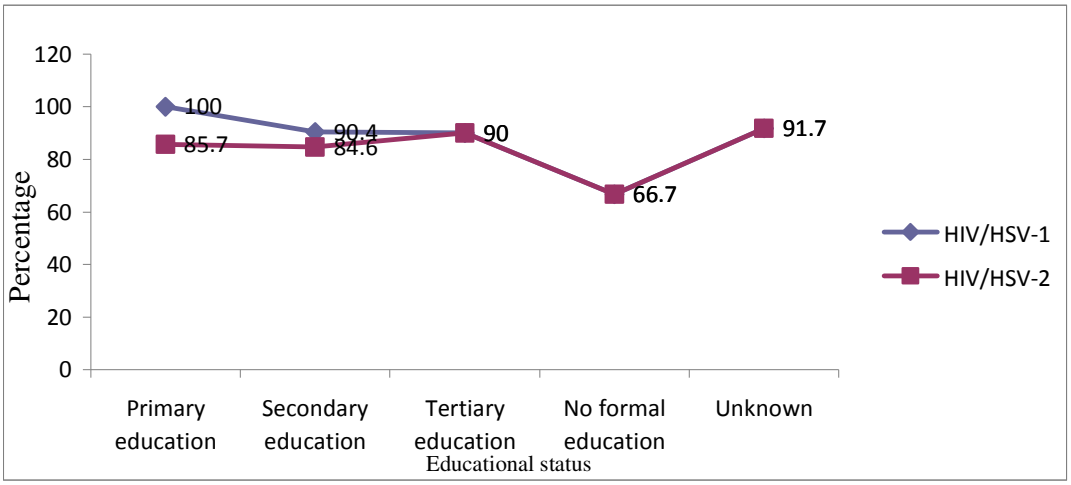


Figure 4: Prevalence rates of HSV-1 and HSV-2 IgM antibodies with respect to educational status of participants

For HSV-1 IgM, those with ≤ 200 CD4 counts/µL (n=54, mean CD4 count=81.6 cells/µL) had lower prevalence rate (88.9% [95% CI: 80.5-97.3%]) compared to 92.2% (95% CI: 87.5-96.8%) among those with > 200 CD4 counts/µL (n=128, mean CD4 count=583.1cells/µL) (Figure 5); the analysis was statistically invalid. For the HSV-2 IgM, there was no significant difference (p=0.9) in the 85.2% of those with ≤ 200 CD4 counts/µL and 85.9% of those with > 200 CD4 counts/µL.

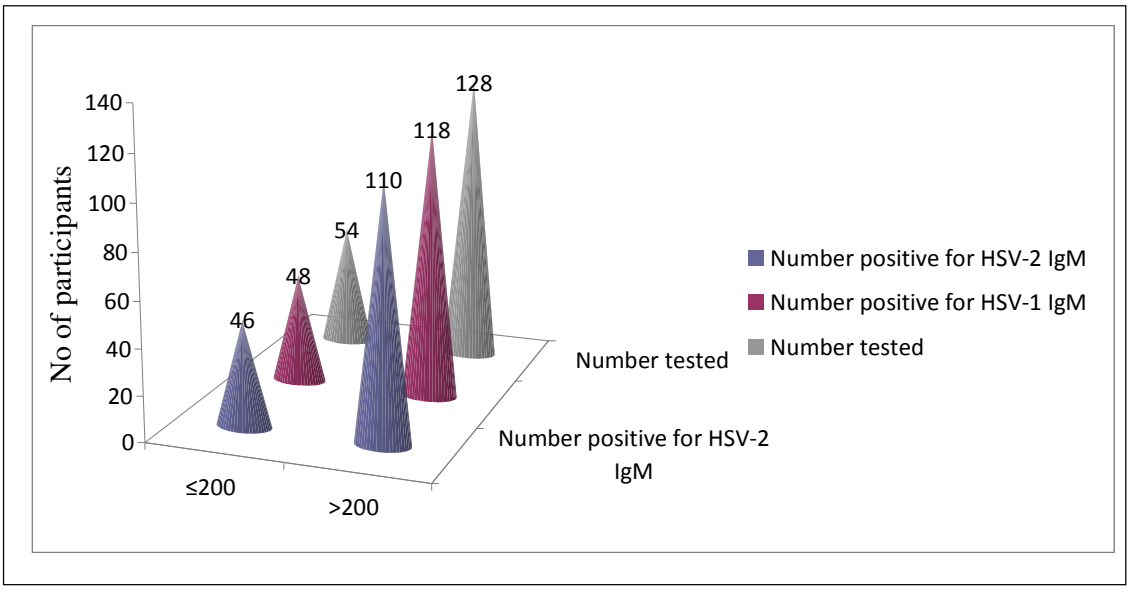


Figure 5: Absolute number of participants positive for HSV-1 and HSV-2 IgM antibodies based on $CD4^+$ count

DISCUSSION

This cross-sectional study investigated exposure of asymptomaticHIV-1 infected participants on ART to HSV-1 and -2 with a view to determining prevalence of the viral infections, and to establish demographic factors associated with HSV IgM prevalence among this cohort of HIV-infected individuals.

HSV infections are latent in nature with persistence in neuronal cells (especially in trigeminal and sacral ganglia) and are frequently reactivated with or without clinical manifestations (Buxbaum *et al*., 2003). Hence, once infected by either HSV-1 or -2, one remains infected for life. Barnabas and Celum (2012) reported that HSV-2 has near constant reactivation and viral shedding which is not completely suppressed even in the presence of standard antivirals. Infection of humans with HSV-2 (and HSV-1) can predispose to HIV-1 infection and *vice-versa* as the viruses can be acquired as STIs. Actually, HIV infections, age at first sexual intercourse, unprotected sexual intercourse and female gender, among others, have been identified as risk factors for acquiring HSVs (Tideman *et al*., 2001, Wald and Link, 2002; Wald, 2004).

Virus-specific IgM is indicative of on-going infection and possibility of infecting susceptible individuals; but for latent viruses like HSV-1 or -2, the IgM seropositivity might be due to primary or recurrent episode. Though this study did not set out to ascertain primary or recurrent infection, in either episode, HSV-IgM positive individual is infectious to susceptible persons if effective oral or unprotected sexual contact occurs. While ELISA for HSV-1/-2 IgM is less diagnostic, it is indicative of burden of on-going infection (Tada and Khandelwal, 2012); and by implication, indicative of infectiousness of seropositive individuals.

Since all the participants in this study were HIV-1 infected, IgM seropositivity to either or both HSVs meant dual or triple infection. That 81.3%, 91.2% and 85.7% had triple infection, HSV-1/HIV-1 dual and HSV-2/HIV-1 dual infection respectively reflect high burden of on-going HSV infections among the ART participants. Also, though prevalence rate of HSV-1 IgM was higher than that of HSV-2 IgM among the participants, that the prevalence rates were statistically comparable points to the fact that the two virus types are similar in nature, behaviour and epidemiology and was suggestive of equal or common exposure of the participants to the HSVs. Though we did not know which virus was superimposed on the other during infection of the study participants, since HIV-1 or HSV-2 infection can mutually predispose to each other (Kamali *et al*., 1999; McFarland *et al*., 1999; Wald and Link, 2002; Freeman *et al*., 2006); the high prevalence rates in this study might be possible or expected. The observation that only 4.4% of the participants were negative to both HSVs points to consideration of including anti-HSV drugs in routine ART to suppress viral shedding and recurrence among ART patients in the study hospital in Imo State.

The high prevalence rates and possible acquisition of both HSV-1 and -2 as STIs point to possibility of the participants engaging in a specific risk factor, possibly unprotected sexual intercourse, at least, in relation to high prevalence of HSV-2 IgM. This is partly supported by the observation that the HIV-1 infected participants were more exposed to HSV-1 and -2 than either virus as shown by 81.3% of dual infection compared to 9.9% of single HSV-1 or 4.4% single HSV-2 infection (Figure 1). This observation is especially of considerable implication in the study locality as all the participants were asymptomatic during sampling. A previous study reported strong and synergistic relationship between HSV-2 and HIV-1 infections; that HSV-2 infection increased the risk for HIV-1 acquisition by two to three folds and that standard prophylactic dose of HSV-2 therapy did not prevent HIV-1 acquisition (Johnston *et al*., 2011; Barnabas and Celum, 2012).

It is noticeable that the study participants were skewed toward females; this supports earlier observation that more Nigerian adult females seek medical care than men (Uneke *et al*., 2005). It was therefore not unexpected that almost 80.0% of the study participants were females. In order to investigate possible association of gender with HSV IgM, we compared the prevalence among male and female HIV-1 participants. In spite of having more females than males, the prevalence rates of HSV-1 IgM among the male and female HIV-1 patients were statistically comparable ($p=0.39$). However, HSV-2 IgM prevalence was associated ($p=0.02$) with gender with the HIV-1 infected females having about three times higher likelihood (OR of 2.9) of testing positive to HSV-2 IgM antibodies compared to their male counterparts.

Arama *et al*. (2010) reported that women might be more susceptible to HSV-2 than men as is the case for HIV infection (Burger and Weiser, 2001). Some suggested reasons for female predisposition to infection include tendency of women to choose sex partners who are older than themselves and likely to have more sexual experience (Mertz *et al*., 1992; Lazcano-Ponce *et al*., 2001). In line with this, higher prevalence of HSV-2 infection has been reported among females in different countries (Allen and Das, 2004; Anuradha *et al*., 2008; Muiru *et al*., 2013; Jacob *et al*., 2015).

Since HSVs can be sexually transmitted, we compared the results of IgM seroprevalence between the married (males and females) and single (males and females) HIV-1 infected participants. We observed that the seroprevalence was generally high in both groups ranging from 85.0% to 100.0%. A possible reason for this could be due to HIV-infected status of all the participants with mutual predisposition between the HSVs and HIV. The HSV-1 IgM prevalence was higher in both groups than that of HSV-2 IgM with all the 22 single participants (100.0%) being positive (Figure 2). Noticeable is the observation that HSV-1 and -2 IgM prevalence rates were higher in the singles (who were 22 in number) than the married (n=160), but the statistical analysis was however, invalid because of zero value for the IgM negative among the singles.

Age, though at first sexual intercourse, is associated with acquisition of HSVs (Cowan *et al*., 2002; Ghebremichael *et al*., 2009). While we did not collect data on the age at first sexual intercourse from the study participants (a limitation of this study); we also observed independent association of age-group 21-30 years with HSV-1 IgM with this age-group having more than five times likelihood (OR: 5.6) of being HSV-1 IgM positive compared to those within age group 31-40 yrs with the lowest prevalence rate (Figure 3). With HSV-2 IgM however, more age groups had independent association with the prevalence; the age-groups 21-30 years, 31-40 years and 41-50 years each had significantly higher HSV-2 IgM prevalence compared to those with unknown ages. Similar trends in prevalence rates have been widely reported (McFarland *et al*., 1999; Smith and Robinson, 2002; Msuya *et al*., 2003; Arama *et al*., 2010; Njuguna *et al*., 2012; Jacob *et al*., 2015). The inconsistencies observed in the trends for both HSVs suggest that more than one factor could be responsible and therefore calls for further research on the natural history of HSV infections among HIV-infected.

Formal educational status can be used as correlate of level of enlightenment regarding acquisition of STIs such that one would expect lesser prevalence rates among humans with tertiary education. Therefore we analysed educational status of the HIV-1 participants *vis-a-vis* their HSV-1 and -2 IgM prevalence rates. Though this variable had no statistical association with the HSV-IgM prevalence rates; the higher rates among those with less than tertiary education is similar to previous reports of strong correlation between lesser education and increased likelihood of infection (McFarland *et al*., 1999; Njuguna *et al*., 2012).

Since $CD4^+$ count is acceptable as indicator of immunologic deficiency or proficiency, we investigated differences between HSV-1 and -2 IgM prevalence rates in relation to $CD4^+$ count of the HIV-1 infected participants. The unexpected lower rate of HSV-1 IgM in those having ≤ 200 $CD4^+$ counts/uL compared to those with > 200 $CD4^+$ counts/uL was puzzling as one would expect those with almost "crashed" $CD4^+$ count to have higher rate. Ditto for HSV-2 IgM. This result is consistent, however, with other studies that found no association between $CD4^+$ count and seropositivity of HSV-1 and -2 among HIV-infected persons (Santos *et al*., 2006; McMahon *et al*., 2011; Tan *et al*., 2014; Jacob *et al*., 2015).

CONCLUSION AND RECOMMENDATIONS

The high prevalence rates of both HSV-1 and -2 IgM antibodies recorded in this study is burdensome on HIV-1 infected individuals and might be a reflection of endemicity of Imo State, Nigeria for the HSVs. The fact that the HIV-1 infected participants were on ART pointed to little or no effect of antiretroviral drugs on HSVs, hence the need to include antivirals specific for HSVs in the regimen for ART. Mass education of the general public on the need and ways of preventing HSVs and HIV infections should be intensified in Imo State and Nigeria as a whole.

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