



doi: <http://dx.doi.org/10.19240/njpas.2017.B06>

**Serosurvey of Herpes Simplex Virus type-2 infection among HIV Infected Individuals Accessing a
Secondary Health Care Facility in Kwara State, North Central Nigeria**

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Abstract

Herpes Simplex Virus type 2 (HSV-2) is a commonly opportunistic infection among HIV seropositive subjects and acts synergistically with HIV to accelerate disease progression. This is across sectional, Hospital-based study designed to determine the prevalence of HSV-2 IgM and IgG antibodies among HIV-1infected individuals accessing the HAART Clinic of General Hospital Offa, Kwara State Nigeria. Blood samples were collected from consenting 276 (age range 11-70; mean age of 36.8 years) HIV-1 infected individuals after collecting pertinent socio-demographic data using questionnaires. The blood was separated into plasma and packed cells. The former was tested for the presence of anti- HSV-2 IgM and IgG using ELISA test kits from Diagnostic Automation INC., USA. Data were analyzed using Chi-square test with SPSS version 21 software and the level of significance was determined to be $P < 0.05$. Out of 276 samples tested, 116 (42.0%) and 39 (14.1%) were respectively positive for HSV-2 IgG and IgM antibodies. These respectively represent latent and active HSV-2 infections. Gender, occupation and clinical state of the subjects were the main correlates of IgM serpositivity while gender, occupation, marital status and CD4⁺ count correlates with IgG seropositivity. The prevalence of anti-HSV-2 IgM and IgG antibodies in this cohort of HIV-infected individuals is high. There is therefore the need for routine test for HSV-2 infection among HIV infected individuals to determine the extent of infection and proper management especially the active infections.

KEY WORDS: HSV-2, HIV, Serosurvey, ELISA, Offa, Antibodies.

Introduction

Genital herpes infection is the primary cause of genital ulcer disease (GUD) (WHO, 2001). Worldwide it is mainly caused by Herpes Simplex Virus type2 (HSV-2). Herpes simplex virus (HSV) is neurotropic with a large linear, double-stranded DNA genome protected by a capsid. The icosahedral capsid is surrounded by an envelope consisting of a lipid bilayer with embedded glycoproteins. Between the capsid and envelope is a proteinaceous region called tegument (Grunewald *et al.*, 2003). HSV-2 is a

member of the Herpesviridae family, subfamily Alphaherpesvirinae, and genus Simplex virus (Anzivino *et al.*, 2009; Straface *et al.*, 2012). This virus has a very complex life cycle and stands out as one of the most common pathogens in the etiology of sexually transmitted diseases worldwide (Pena *et al.*, 2010). HSV-2 infection is sexually transmitted and more prevalent in adults and can be transmitted by both symptomatic and asymptomatic individuals (WHO, 2001).

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Owing to its morbidity and frequency of recurrence, genital herpes disease is of particular public health importance.

HSV-2 infection is said to be a significant factor in the increased risk of the acquisition and transmission of human immunodeficiency virus (HIV) (Glacier *et al.*, 2006). There is considerable biologic and epidemiologic evidence that HSV-2 infection is a cofactor for both acquiring and transmitting HIV (Des Jarlais *et al.*, 2010). Two metaanalyses and a qualitative review have made a submission that prevalent HSV-2 infection is associated with an increased likelihood of acquiring HIV by a factor of 2 to 3 (Freeman *et al.*, 2006; Tobian and Quinn, 2009). At the biologic level, HSV-2 may create lesions that may serve as entry portals for HIV while simultaneously recruiting target cells (dendritic and CD4⁺ cells) for HIV infection to the genital mucosa (Des Jarlais *et al.*, 2010). HSV-2 infection also increases HIV RNA in genital secretions and may also increase transmission of HIV (Van *et al.*, 2008). The reciprocal enhancement of HSV-2 and HIV viral replication in the presence of concurrent infection has also been documented (Henget *et al.*, 1994; Brugha *et al.*, 1997). It might also contribute to HIV transmission because genital ulcers may bleed during intercourse, leading to an increased risk of HIV transmission. It has been suggested that genital ulcerative disease may increase infectiousness among HIV infected people with GUD, since HIV proviral DNA has been detected in genital ulcer exudates (Mbopi-Keou *et al.*, 2003).

Most research on the relationships between HSV-2 and HIV has been conducted in sub-Saharan Africa, and it has been estimated that HSV-2 may account for 35% to 48% of HIV infections in various sub-Saharan African cities (Freeman *et al.*, 2007). The first documented isolation of HSV-2 in Nigeria was done in Ibadan (Oni *et al.*, 1994). The HSV-2 seroprevalence rate among female sex workers in Lagos, Nigeria was 59% (Dada *et al.*, 1998). Higher HSV-2 prevalence was reported in a multi-centre study of sex workers in four sub-Saharan African cities (Lagarde *et al.*, 2001).

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 and Link, 2002).

Genital herpes is not routinely diagnosed in the Hospital laboratories among HIV- Infected individuals in Nigeria. Also, there is dearth of information on the HSV-2 seroprevalence and its association with HIV infection in North Central Nigeria, particularly in Offa Kwara State. The present study is aimed at determining the prevalence of HSV-2 IgG and IgM antibodies and identifying probable risk factors among HIV subjects attending the HIV Clinic of Offa General Hospital, Kwara State Nigeria.

Methodology

Study design

This is a cross-sectional, hospital-based serosurvey. Approval to carry out the study was obtained from Health Ethical research committee of the Kwara state Ministry of Health.. Blood specimens were obtained with informed consent from 276 HIV-infected individuals aged between 11-70 years who were attending the HAART Clinic of Offa General Hospital, Kwara State. A structured questionnaire was used to obtain demographic and behavioural information from each patient.

Description of analysis

About 5ml of venous blood samples were collected from each subject into sterile anticoagulant-free bottles and allowed to clot. The samples were spun at 3000 rpm for 5 min and serum aspirated into sterile cryovials. The samples were properly labelled and stored at -20°C until assayed. The sera which tested for HSV-2 (IgM and IgG) antibodies, using commercial ELISA kits (The Diagnostic Automation ELISA.; Manufacturer Diagnostic Automation, INC.; 21250 Califa Srt, Suite 102 and 116, Woodlands Hills, Ca 91367, USA). All the steps were performed according to the manufacturer's instructions. Absorbance was read at 450nm wave length with Micro-pate reader (Sigma Diagnostic). To fix the cut-off ranges, the mean absorbance value of the supplied standard serum (STD) was multiplied with the numerical

data quality control provided by the manufacturer, for example, $OD = 0.502 \times MW$ (STD) with upper cut-off and $OD = 0.352 \times MW$ (STD) with lower cut-off.

Page | 3061 **Statistical Analysis**

Data were subjected to statistical analysis using the software SPSS version 21 (SPSS Inc., Chicago, USA). Pearson's chi-square test was performed at 95% confidence interval. P values < 0.05 were considered statistically significant.

RESULTS

Of the 276 samples tested, 39 (14.1%) and 116 (42.0%) were seropositive for HSV-2 IgM and IgG antibodies, respectively (Table 1). The positivity for HSV-2 IgM indicates current or recent infection with HSV-2, while the positivity for HSV-2 IgG indicates past infection with HSV-2.

Samples were collected from 62 (22.5%) males and 214 (77.5%) females (Table 1). The prevalence of HSV-2 IgM antibody was 22.6% (14/62) and 11.7% (25/214) among males and females, respectively, while the prevalence of HSV-2 IgG was 61.3% (38/62) and 42.0% (78/214) among males and females respectively (Table 1). Statistical analysis showed a significant correlation between gender of the tested subjects and the presence of HSV-2 IgM and IgG antibodies (P = 0.004 and 0.0008 respectively)(Table 1).

The prevalence of the infection appeared to have increased with age among both males and female

subjects. Highest prevalence (22.2%) of HSV-2 IgM antibodies in this cohort was recorded among study participants of age range 21-30 years. Also, the highest prevalence (59.3%) for HSV-2 IgG antibodies was also recorded among participants of this same age range. Lowest prevalence (8.0%) of IgM antibody and (16.7%) of IgG antibody respectively was recorded among subjects of age range 11 - 20 years. Highest prevalence (28.6%) of HSV-2 IgM antibody was recorded among students. Also, the highest prevalence (50.8%) of HSV-2 IgG antibody was recorded among the traders. Lowest prevalence (14.3%) of IgG antibody was found among the professionals while IgM antibody was not detected among the professionals in the study group (Table 1). Statistical association showed a significant correlation between the presence of IgG antibody and marital status of the subjects (P value = 0.003). Of the 63(22.8%) single individuals tested, 15.9% and 33.3% were positive for IgM and IgG antibodies respectively and of the 182(65.9%) married individuals tested, 15.4% and 48.9% were positive for both IgG and IgM respectively (Table 1). Among this cohort also, HSV-2 infection appeared to be highest among the widowed with 16.7% and 66.7% recorded for IgG and IgM antibodies respectively. Lowest prevalence of 8.0% of IgG antibody was found among the divorced (Table 1).

Table 1: Relationship between study predictors and detection of anti-HSV-2 IgM/IgG antibodies among the HIV-infected study participants

Predictors	No Tested	No (%) positive			
		IgM	χ^2 (P value)	IgG	χ^2 (P value)
Gender					
Male	62	14 (22.6)	3.85 (0.049)	38 (61.3)	11.18 (0.008)
Female	214	25 (11.7)		78 (36.4)	
Age group (Yr)					
11-20	12	1 (8.0)	3.85 (0.5712)	2 (16.7)	8.165 (0.147)
21-30	27	6 (22.2)		16 (59.3)	
31-40	98	10 (12.4)		36 (36.7)	
41-50	65	9 (10.2)		30 (46.2)	
51-60	43	8 (18.6)		18 (41.9)	
61-70	31	5 (16.1)		14 (45.2)	
Occupation					
Traders	177	21 (11.9)		90 (50.8)	

Civil servants	22	2 (9.1)		8 (36.4)	
Artisans	29	10 (3.4)		5 (17.2)	
Professionals	14	0(0)		2 (14.3)	
Students	7	2 (28.6)		3 (42.9)	
Undetermined	27	3 (11.1)	15.12 (0.009)	8 (29.6)	19.38 (0.002)
Marital status					
Single	63	10 (15.9)		21 (33.3)	
Married	182	32 (15.4)		89 (48.9)	
Divorced	25	0 (0)		2 (8.0)	
Widowed	6	1 (16.7)	4.54 (0.208)	4 (66.7)	18.9 (0.0003)
Total	276	39 (14.1)		116 (42.0)	

P value < 0.05 statistically significant

Prevalence rate of the participants in relation to ART status was also analysed. No statistical association was observed among those that were ART experienced and those that are ART naïve for both IgG and IgM. Also, the prevalence rate with respect to clinical manifestation of AIDS was also determined, it was observed that 5 participants had signs of full blown AIDS and out of which 3 (60%) and 4 (80%) tested positive for HSV-2 IgM and IgG respectively. However statistical

significance was observed only for HSV-2 IgM antibody (P Value = 0.013) (Table 2).

Prevalence rates in the participants in relation to CD4 counts were also analysed. For HSV-2 IgM those with ≤ 350 cells/ μ L had lower prevalence rate (12.4%) while those with >350 cells/ μ L had a higher prevalence (18.3%); the analysis was statistically insignificant. For HSV-2 IgG there was a statistically significant difference (P Value = 0.016) between the 71.5% with ≤ 350 cells/ μ L and the 30.5% with > 350 cells/ μ L (Table 2).

Table 2: Relationship between Therapy/clinical status and detection of anti-HSV-2 IgM/IgG antibodies among the HIV-infected study participants

Predictors	No Tested	No (%) positive			
		IgM	χ^2 (P value)	IgG	χ^2 (P value)
ART status					
Experienced	273	14 (13.9)		116 (42.5)	
Naïve	3	1 (33.3)	0.02 (0.888)	0 (0)	0.8 (0.371)
Clinical manifestation of AIDS					
Yes	5	3 (60.0)		4 (80.0)	
No	271	35 (12.9)	6.13 (0.013)	112 (41.3)	1.64 (0.200)
CD4 count (cells/ μ L)					
≤ 350	194	21 (11.9)		90 (50.8)	
> 350	82	2 (9.1)	1.21 (0.271)	8 (36.4)	5.72 (0.016)
Total	276	39 (14.1)		116 (42.0)	

P value < 0.05 statistically significant

Discussion

Genital herpes is a global problem, it can cause significant distress, and can have a devastating impact on the social and psychological wellness of an individual (Kim *et al.*, 2012). This cross-sectional study investigated exposure of HIV-1

infected participants to HSV-2 with the view to determining prevalence of this viral infections, and to establish demographic factors associated with HSV IgM and IgG prevalence among the cohort.

In this study, of 276 HIV infected subjects it was found that the overall seroprevalence of HSV-2

IgM and IgG was 14.1% and 42.0% respectively. This result is in disagreement with the results obtained in Tamil Nadu, India, where the prevalence of HSV-2 IgM and IgG antibodies in HIV reactive subjects were 28.6% and 60% respectively (Jacob *et al.*, 2015). Also, this result is in disagreement with the HSV-2 IgG prevalence of 87.0% reported in Nigeria by Agabi *et al.* (2010) which was significantly higher. Our findings confirm the high prevalence of HSV-2 infection in Nigeria. It further suggests a strong association between HSV-2 infection and some socio-demographic and clinical parameters of the HIV infected subjects.

In this study, it was noticeable that the study participants were more of females (77.5%). This supports earlier observation that more Nigerian adult females seek medical care than men (Uneke *et al.*, 2005). In order to investigate possible association of gender with HSV-2 IgM and IgG, the prevalence among male and female HIV infected participants was compared. The seropositivity of HSV-2 IgM and IgG was higher in males (61.3%) than in their female (22.6%) counterparts. This was also statistically significant ($P=0.047$ and 0.0008 respectively). This finding is in disagreement with the findings of Jacob *et al.* (2015) who reported a higher seropositivity of HSV-2 IgG in female than in male HIV positive subjects.

In this study, the highest numbers of HSV-2 seropositive subjects were in 21-30 years age group, while the least was recorded in the 11 - 20 years age group. Similar observations were also reported by the earlier workers that, young middle-aged subjects are more sexually active which makes them more vulnerable (Looker *et al.*, 2008). Since HSV-2 is sexually transmitted, the results of IgM and IgG seroprevalence between the married, single, divorced and widowed (males and females) HIV-1 infected participants was compared. It was observed that for both IgG and IgM the seroprevalence was generally higher in the married and widowed group (Table 1). Since the two viruses are sexually transmitted, sexual activities between these two groups may partly explain the observed high prevalence amongst them. A

statistical association was observed in the detection of IgG antibody in the study population and the marital status ($P=0.0003$).

With respect to clinical signs of AIDS, it was observed that 5 out of the 276 HIV positive subjects had clinical presentation of AIDS and out of which 60% tested positive for HSV-2 IgM antibody while 80% tested positive for HSV-2 IgG antibody. There is strong correlation between distributions of HSV-2 IgM antibody with the clinical manifestation of AIDS among the study participants. The higher prevalence found among subjects with clinical signs of AIDS than their asymptomatic counterparts is an indication of immunosuppressed nature of the former group which leads to reactivation of the HSV-2 infection. Since $CD4^+$ count is a known and acceptable indicator of immunologic deficiency or proficiency, differences between HSV-2 IgM and IgG prevalence rates in relation to $CD4^+$ count of the HIV infected participants was investigated. There was a high prevalence of IgG in those having ≤ 350 $CD4^+$ cells/uL and which was statistically significant (P Value = 0.0016). While a higher prevalence of IgM antibody was observed in those having > 350 $CD4^+$ cells/uL. This results are in discordance however, with other studies that found no association between $CD4^+$ count and seropositivity of HSV -2 among HIV-infected persons (Santos *et al.*, 2006; McMahon *et al.*, 2011; Tan *et al.*, 2014; Jacob *et al.*, 2015;).

The results highlight the potential public health impact of HSV-2 in Nigeria where anti-HSV-2 testing is not generally performed in all populations, despite the synergy between HIV and HSV-2 transmission. Testing, identifying and counselling those at risk should have a major impact on reducing the incidence of HSV-2 infection. Thus, this report underlines some public health concerns when placed in the context studies which show that mucosal HSV-2 ulcerations, whether symptomatic or asymptomatic, are associated with influx of $CD4^+$ T cells, which are easily infected with HIV (Koelle and Wald, 2000).

Conclusions and recommendations

There is a significantly high prevalence rate of both HSV-2 IgM and IgG antibodies in the HIV-positive individuals recruited for this study and this is a reflection that HSV is endemic in Offa Kwara State, Nigeria. It is therefore recommended that HSV-2 should also be routinely tested in HIV-infected and non infected persons as this will help elucidate the extent of the infection and enhance management of the infection. Mass education of the general public on the need and ways of preventing HSV-2 and HIV infections should be intensified in Kwara State and Nigeria as a whole.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the following: Kwara State Ministry of Health for granting ethical approval to conduct this research and also the management of the General Hospital Offa for granting us access to the patients in the facility.

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