



SEROPREVALENCE OF HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) INFECTION AMONG HIV PATIENTS ACCESSING HEALTHCARE AT FEDERAL MEDICAL CENTRE, KEFFI, NIGERIA

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ABSTRACT

Herpes simplex virus type 2 (HSV-2) infections is a sexually transmitted infection worldwide, which has a public health implications especially as a driving force behind the Human Immunodeficiency Virus (HIV) epidemic. It is known to cause genital ulcer as well as lesions. It is a lifelong recurrent disease with no cure. Due to the lack of documented HSV-2 studies among HIV patients in this study area with an estimated HIV prevalence of 38.7%, there was a need for estimating the seroprevalence of HSV-2 infection in the study population. The sera of 223 consenting HIV positive patients were screened for HSV-2 specific IgG using an ELISA test kit (Cortez Diagnostic, Inc, USA). The chi-square test was performed to identify possible risk factors associated with the viral seropositivity. The overall seroprevalence of the viral infection was 77.6%. Females recorded a prevalence of 84.5% and males 51.0% ($p > 0.05$). Participants aged ≤ 20 and ≥ 61 years recorded a prevalence of 100%, while the lowest prevalence (66.7%) was observed in those aged 51-60 years ($p > 0.05$). There was a statistically significant association between the seroprevalence of HSV-2 in HIV patients in relation to locality. Patients from the rural setting had a higher prevalence (85.2%) of the infection than those from the urban setting (72.6%) ($p \leq 0.05$). However, in this study, marital status, occupation, level of education, antiretroviral therapy (ART) status and CD4 counts, had no statistically significant association with HSV-2 infection ($p > 0.05$). Awareness campaigns that will promote behavioral change might be the most important strategy to mitigate transmission as most of the infected persons usually show no clinical symptoms. The role of vaccination and condom use among high risk groups may help in combating the transmission.

Keywords: HSV-2, HIV, ELISA, Seroprevalence, Keffi

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Contribution/ Originality

This study has contributed in documenting the prevalence of the infection in Keffi using the ELISA test kit and has used the Chi square statistical test. This study is one of the very few studies which have investigated the prevalence of HSV-2 infection in North central, Nigeria. The paper has contributed in logical analysis and in estimating the prevalence and risk factors of the infection in Keffi. The study documented a very high overall prevalence of the infection. It is associated with gender, age and locality and not associated with marital status, occupation, level of education, ART and CD4 counts.

1. INTRODUCTION

Herpes Simplex Virus type 2 (HSV-2) is one of the most commonly sexually transmitted viruses with humans as their only reservoir [1, 2]. Its prevalence in adult general population in sub Saharan Africa ranges from 30 – 80% in women and 10 – 50% in men [3]. HSV-2 often presents with oral and anogenital ulcers but can also cause serious systematic manifestations especially in neonates where the infection has poor prognosis [4]. After the initial infection, the virus persists for life in a latent form within the neurons of the host from where it causes recurrent episodes when reactivated [5]. Low CD4 counts and high viral loads are associated with increased frequency of HSV-2 reactivation [6] In fact HIV positive individuals are four times more likely than HIV negative individuals to have reactivation of infection and so are more likely to transmit the virus [6].

The ulcerations caused by the viral infection disrupt the genital epithelium and serve as portal of entry for HIV infected genital secretions. These ulcerations also cause an inflammatory response which stimulates more T- lymphocytes around the ulcer. These T-cells are more susceptible to HIV infection than unstimulated cells [7]. Thus this virus is said to play an important role in the dynamics of HIV infection [8]. Many studies have shown that HSV infection increases the risk of HIV infection by at least 2 fold [1]. While Africa has the highest prevalence of infection, in Sub Saharan Africa more than 85% of HIV-1 infected individuals are HSV-2 positive and by the age of 25 years half of the general population is HSV-2 seropositive. Worldwide, the prevalence of HSV-2 among HIV positives is 52 -95% [9].

There is a dearth of information on the prevalence of HSV-2 among HIV patients in this study area. The present study therefore mooted as a baseline study to determine the prevalence of HSV-2 infection and the probable risk factors among HIV positive patients accessing healthcare in Keffi.

The information will serve as a tool for the establishment of intervention strategies that will help in reducing the rates of HSV-2 related morbidity and mortality in HIV patients.

2. MATERIALS AND METHODS

2.1. Study Area and Population

The study area for this research was Keffi. It is approximately 68km from Abuja the Federal Capital Territory and 128km from Lafia the capital of Nasarawa State. Keffi is located between latitude 8°5'N of the equator and longitude 7°8'E and situated on an altitude of 850m above sea level [10].

The study was carried out among 223 adults living with HIV infection and accessing healthcare at Federal Medical Centre, Keffi, who agreed to participate in the study. The socio-demographic information of the participants was obtained by use of oral interview, while their CD4 T-cell counts were retrieved from their hospital record files.

2.2. Sample Collection

About 5ml of blood sample was collected by venepuncture from all the enrolled subjects in a sterile plain universal container. The blood sample was allowed to clot for 30 minutes and centrifuged at 3000rpm for 5 minutes. A Pasteur pipette was used to harvest and dispense each serum into a new, labeled plain tube. The samples were transported in a cold box to Innovative Biotech Ltd Laboratory, Keffi and stored at -20°C until ready for use.

2.3. Laboratory Investigation

The HSV-2 specific IgG ELISA test kit (Cortez Diagnostic, Inc. USA) was used to detect HSV-2 IgG antibodies in the sera according to the manufacturer's instructions.

2.4. Enzyme Linked Immunosorbent Assay (ELISA) Procedure

The serum was thawed at room temperature. All the reagents and microtitre plates were also equilibrated to room temperature. HSV-2 antigen microtitre wells coated strip was placed into a holder and 1:40 dilution each sample, negative control, positive control and calibrator was prepared by adding 5µl of the respective serum to 200µl of sample diluent. It was mixed by gentle agitation of the plates. One hundred µl of diluted serum, calibrator, and controls was dispensed into an appropriate well. For the reagent blank, 100µl sample diluent was dispensed into 1A well position. The holder was tapped gently to remove air bubbles from the liquid and to mix contents of each well. The plate was incubated for 30 minutes at room temperature. The liquid was poured out from all wells and the microtitre strip dapped on tissue. The wells were washed 3 times with 200µl of washing buffer each time. After the third wash, 100µl of enzyme conjugate was dispensed into each well and the strip incubated for 30 minutes at room temperature. The enzyme conjugate was then removed by pouring out into a container containing some disinfectant and further dapped onto a tissue paper pad and washed again. The washing was repeated 3 times with washing buffer and 100µl of TMB chromogenic substrate was dispensed into each well and the strip was incubated for 30 minutes at room temperature. After the incubation period 100µl of 2N HCl was added to stop reaction. The result was read at an Optical Density (O.D) of 450nm using a microwell ELISA reader (BIO-RAD PR2100) [11].

2.5. Ethical Approval

The study was conducted with the approval of the Health Research Ethical Committee (HREC) involving humans of the Federal Medical Centre, Keffi.

2.6. Statistical Analysis

Data obtained from the study were analyzed using Chi-square (χ^2) to determine the association of the seroprevalence of herpes simplex virus type 2 (HSV-2) infections among HIV patients with the studied risk factors. Values obtained were considered statistically significant at $p \leq 0.05$.

Table-1. Prevalence of HSV-2 among HIV patients in Keffi with respect to some sociodemographic variables

Variables	No Examined	No. Positive (%)	P value
Sex			> 0.05
Male	49	26(53.0)	
Female	174	147(84.5)	
Marital Status			> 0.05
Married	137	110(80.3)	
Single	86	63(73.3)	
Occupation			> 0.05
Students	59	40(67.8)	
Civil Servants	21	13(61.9)	
Farmers	17	10(58.8)	
Housewives	36	29(80.6)	
Artisans	90	81(90.0)	
Education			> 0.05
≤ Primary	55	46(83.6)	
Secondary	116	94(81.0)	
Tertiary	52	33(63.5)	
Locality			≤ 0.05
Urban	88	75(85.2)	
Rural	135	98(72.6)	

3. RESULTS

A total of 223 HIV positive patients were recruited for this study. Of these 22% were males and 78% were women. There was an overall HSV-2 prevalence of 77.6% with 84.5% among females and 51.0% among males

(Table 1) ($p > 0.05$). Educational level attained, marital status and occupation were not significantly associated with the viral prevalence ($p > 0.05$) but locality was significantly associated with infection ($p \leq 0.05$) as shown on Table 1.

Similarly, age, HAART status and CD4 counts level of the participants did not show a statistically significant association with infection (Table 2).

Table-2. Prevalence of HSV-2 infection among HIV patients in Keffi with respect to some variables

Variables	No Examined	No. Positive(%)	P value
Age (years)			> 0.05
≤ 20	19	19(100)	
21 – 30	125	90(72.0)	
31 – 40	54	43(79.6)	
41 – 50	18	16(88.9)	
≥ 51	7	5(71.4)	
HAART Status			> 0.05
On HAART	158	115(72.8)	
HAART naïve	65	58(89.2)	
CD4 Count			> 0.05
≤ 200	37	32(86.5)	
201 – 300	31	27(87.1)	
301 – 400	47	42(89.4)	
401 – 500	52	43(82.7)	
501 – 600	20	10(50)	
≥ 601	36	19(52.8)	

4. DISCUSSION

The overall seroprevalence of 77.6% HSV-2 infection recorded in this study is relatively high especially when compared to similar studies carried out in other parts of Nigeria. There have been reports of 24.4% [12] and 9.7% in people reporting for counseling and testing. An earlier report of 59% from commercial sex workers [13] and a more recent study reported 87.0% [3] among HIV infected individuals. Lower rates compared to findings in the present study have also been reported in other countries like 4.0% from HIV positive children in Malawi [4] the high prevalence of HSV-2 infection reported among HIV patients confirms their shared routes of transmission and the possibility of being infected with the other when infected by one. It also supports the outcome of some research that showed that there is an association between HIV and HSV-2 infection.

The higher seroprevalence of HSV-2 infection among females (84.5%) than their male counterparts (51.0%)($p > 0.05$) reported in this study is similar to another Nigerian study but contrasts another [3]. Reports from United States [1] India [6] Canada [14] and Brazil [7] all showed a female dominated prevalence of HSV-2 infection although with different rates. This might have resulted from difference in sample size, and characteristics of the study population. However, this higher prevalence is also a confirmation of their shared route of transmission with HIV which is globally higher among women. Just like the case of HIV, this higher rate in women has been ascribed to biologic and behavioral factors which include the possession of a larger mucosal surface area of the female genital tract which is prone to infection and the receptive role of women in the act of sex with a consequent higher male-to-female transmission risk per exposure. Sexual contact with older partners who are more likely to be HSV-2 seropositive has also been suggested [6]. The distribution of HSV-2 infection when stratified by age appeared to be high in all the age groups and there was no statistically significant association between age and the viral infection ($p > 0.05$). The least infected group (71.4%) was people aged 51 years and above. Comparatively the sample size of members of this age group was quite small. The general high prevalence in all the age groups must have been because of high sexual activities within those age groups. Agabi, et al. [3] made a similar observation although it was not significantly associated with infection but in other studies it was found to be a risk factor [6, 15, 16]. With

the dynamics of the co-infection of HIV and HSV-2, the possibility of many of the older people having died cannot be ruled out. Infection with the 2 viruses is a life-long health challenge [5] that sometimes people from this study area fail to accept the medical explanation but would rather go for traditional healing thus such people could not have been available for this study. It is very likely that the older ones would have been the ones that had been suffering for a longer period so might be the ones that will go for traditional healing thus depleting their numbers. The paucity of samples among those aged ≤ 20 years (which does not include those aged 0 – 16 years) could have led to the 100% prevalence recorded although heightened sexual activity at this tender age cannot be ruled out as it is the time of experimenting life and females might even be married at that age in this part of Nigeria and with HSV-2 transmitted mainly sexually such high prevalence is possible. Similarly the viral infection was not associated with marital status. There was a prevalence rate of 80.3% among those that are married and 73.3% among the singles. This might not be unconnected with the fact that both viruses are mainly sexually transmitted and risky behavior is likely to have predisposed the participants to infection by any one of the viruses (HIV or HSV-2) which could have paved way for an infection by the other. It has been observed that in sub-Saharan Africa sometimes HIV infection can be attributed to HSV-2 infection [5].

With reference to occupation, artisans recorded the highest seroprevalence (90%), followed by housewives (80.6%), students (67.8%), civil servants (61.9%) and the least seroprevalence was recorded among farmers (58.8%). There was no statistically significant association between viral infection and occupation. In a related development, viral seroprevalence was highest among those with the lowest level of education and lowest among those with a tertiary education ($p > 0.05$) but with consistently high prevalence irrespective of educational level attained. Education has been acknowledged to be of advantage in various facets of life. It helps in making informed decision and also sourcing for useful information regarding health concerns. However, prevalence still remained consistently high irrespective of educational status. An earlier review had posited that for HSV-2 seroepidemiology, socio-economic status, religion and educational status have no significant effect on the viral infection [17].

There was a statistically significant association between locality and the viral infection ($p \leq 0.05$). The prevalence of HSV-2 infection was higher among rural (85.2%) than the urban participants (72.6%). This was expected in view of the relationship between HIV and HSV-2 prevalence. Some researchers have reported the higher prevalence of HIV in rural participants than urban participants [18] it is therefore logical to expect the same trend for HSV-2. Others have noted that there were more HSV-2 seropositives from the rural population than the urban [19].

There was no statistically significant association between antiretroviral treatment status and the viral infection ($p > 0.05$), the prevalence of infection was higher among HAART naïve (89.2%) than those on HAART (72.8%). Similarly, there was no statistically significant association between CD4 counts and the viral infection among the study participants ($p > 0.05$). Although low CD4 counts and high viral loads are known to be associated with increased frequency of HSV-2 reactivation [6] some earlier workers had posited that HSV-2 infection is not affected by CD4 count level or being on HAART or not [20].

5. CONCLUSION

A HSV-2 prevalence of 77.6% was reported in the present study. This finding confirms the high prevalence of HSV-2 among HIV patients in Keffi. The recognition of this burden of infection in HIV patients' calls for routine screening for HSV -2 infection in such patients. Most patients are asymptomatic to HSV-2 infection so serve as a reservoir of transmission of the virus to uninfected people. Therefore the creation of awareness through Health Education among high risk groups will play key role to limit the infection.

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