Seroprevalence and Molecular Detection of Herpes Simplex Virus Type-2 (HSV-2) among People living with HIV/AIDS in Northwestern, Nigeria

Ibrahim Abubakar Muhammad
Bayero University Kano (BUK)
Taysir Ramadan Hafiz
Bayero University Kano (BUK)
Faisal Muhammad
Daffodil International University (DIU)
Rine Christopher Reuben
German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig
TasiuAdamu Sani
Ahmadu Bello University Zaria (ABU)
AbubakarSunusi Adam
Federal University Dutsin-Ma (FUDMA)
LawalDahiru Rogo (ldrogo.med@buk.edu.ng)
Bayero University Kano (BUK)

Research Article

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Abstract

Background

In most developing countries, including Nigeria, herpes simplex virus type 2 (HSV-2) is associated with an increased risk of HIV acquisition and transmission, which often results in more frequent, lasting, and severe clinical outcomes. Despite the association between HSV-2 and HIV, knowledge regarding HSV-2 among people living with HIV/AIDS (PLWHA) in northern Nigeria is elusive.

Methods

This cross-sectional study sought to determine the seroprevalence and molecular detection of HSV-2 among PLWHA attending a referral hospital in Northwestern Nigeria. Blood samples collected from 180 PLWHA were screened for HSV-2 IgM using Enzyme-Linked Immunosorbent Assay (ELISA) and then subjected to molecular characterization using HSV-2 specific PCR. Moreover, socio-demographic data and risk factors of the sampled population were collected using a structured questionnaire.

Results

The overall seroprevalence of HSV-2 was 6.1%, with 5.0% and 1.1% in females and males, respectively. However, no significant association ($P > 0.05$) existed between HSV-2 seroprevalence with marital status, gender, occupation, residence, educational level, age, history of sexually transmitted diseases (STDs), ethnicity, and the number of sex partners. In addition, condom use significantly ($P < 0.05$) reduced the risk of HSV-2 infection among the study population. However, only 5 (45.45%) of the 11 (100.0%) HSV-2 seropositive subjects were molecularly confirmed to be HSV-2 positive using PCR.

Conclusion

This is the first study to confirm the presence of HSV-2 infection among PLWHA in Northwestern Nigeria. Data obtained stress the need for surveillance of HSV-2 therapy, and public enlightenment on the use of condoms to reduce the risk of HIV transmission.

1. Introduction

In Nigeria and other developing countries, herpes simplex virus type 2 (HSV-2) is one of the most common causes of genital ulcers. It is also associated with an increased risk of HIV acquisition and transmission, resulting in more frequent, lasting, and severe clinical outcomes. In addition, there is a direct relationship between STIs and genital HIV shedding in people living with HIV/AIDS (PLWHA) [1], and such co-infections may facilitate the sexual transmission of the virus and accelerate disease progression [1–3].

Africa has the highest prevalence of HIV infection [4, 5]. It was established that more than 85% of people living with HIV in sub-Saharan Africa were HSV-2 seropositive [6]. In 2016, an estimated 491.5 million

people were living with HSV type 2 infections; this is equivalent to 13.2% of the world's population aged 15–49 years [7]. The prevalence of HSV-2 in Nigeria is 24.4% [8], and another study found a 36.4% prevalence of HSV-2 among HIV seropositive patients in Abuja, Nigeria [9]. Moreover, for HSV-2/HIV coinfected individuals, the shedding of HIV is common, resulting in the risk of HIV transmission to sex partners [10]. Modeling studies have demonstrated that HSV-2 plays a significant role in triggering the spread of HIV in Sub-Saharan Africa [11].

The prevalence of HSV-2 co-infection rates among HIV-infected individuals is not widely reported. Most studies focus on the seroprevalence of HSV-2 IgG or IgM only, without going further from serology to confirmation by molecular technique that is more sensitive and reliable. HSV-2 coinfection in HIV patients is a risk factor for developing a secondary genital infection that can be life-threatening in HIV individuals and enhance the infectiousness of HIV-positive subjects and susceptibility to HIV-negative subjects with an effect on genital HIV shedding [12]. Despite the significant risk of HSV-2 associated with PLWHA, the epidemiology of this coinfection is poorly defined in Nigeria, especially the Northern region with a higher population, malnutrition, food insecurity, and disease burden [13]. Moreover, the anti-HSV-2 screening test is routinely not performed in Nigeria. However, this may serve as a pointer in identifying co-infection among PLWHA having a higher risk of HIV transmission to their sexual partners or even vertically, from mother to child. Generally, there is a lack of information on the prevalence of HSV-2 among people living with HIV/AIDS (PLWHA) and associated socio-demographic risk factors in Nigeria. Therefore, it sought to determine the seroprevalence and molecular detection of HSV-2 and associated risk factors among (PLWHA) in a reference hospital in northwestern Nigeria. This will open new horizons for our understanding of the current epidemiological dynamics of HSV-2 among the population, especially PLWHA, and then inform a decision on the management of HSV-2, HIV, and other STIs.

2. Methods

2.1 Study design, population, and size:

The study was a descriptive cross-sectional study. The samples were obtained using a simple random sampling technique from people living with HIV/AIDS (PLWHA) attending Aminu Kano Teaching Hospital, Kano, a major regional HIV referral hospital in Northwestern Nigeria. The sample size was determined based on the prevalence of 7.8% [14] with a 95% confidence level and 5% precision [15]. A total of 180 PLWHA were sampled for this study between August 2019 to March 2020.

2.2 Sampling:

Five (5) milliliters of blood sample were collected aseptically from each participant by venipuncture and transferred into a labeled plain sterile vacutainer tube. The collected blood sample was then allowed to clot at room temperature, and the sera were also separated by centrifugation at 3000rpm for 10 minutes and kept at -20 until needed for analysis. Moreover, a structured questionnaire was administered to each consented participant during the period of the study for socio-demographic data as well as risk factors associated with HSV-2.

2.3 Serological Assay:

According to the manufacturer's instructions, Sera were analyzed using Enzyme-Linked Immunosorbent Assay-ELISA (Elabscience Biotech, Wuhan China). Briefly, one well was set as a blank control, three wells for the negative control, and two wells for positive control. Fifty microlitres (50μL) of serum was added to each well, and the blank control was kept empty, and then the plate was shaken gently to ensure thorough mixing. The microtiter plate was covered with a plate sealer and then incubated for 30 minutes at 37ºC. After incubation, washing was done up to five times with wash buffer and then immersed for 30–60 seconds each time. Fifty microlitres (50μL) of HRP conjugate was added to each well except the blank control well and subsequently incubated for 30 minutes at 370C. Washing was repeated up to five times, and then 50μL of substrate A and substrate B were added,respectively, and incubated for 15 minutes at 370C in the dark. Finally, fifty microlitres (50μL) of stop solution was added to each well, and the plate was gently tapped to ensure thorough mixing. The color intensity corresponds to the amount of HSV-2 antibody in the specimen, which was measured using a microplate reader at 450nm in which the value of each well was detected.

2.4 Molecular Detection:

According to the manufacturer's instructions, the extraction of viral DNA was performed using 300μL of serum obtained from individuals participating in the study with the DNA Extraction mini kit (Norgen, Biotek; Canada). A pair of forward and reverse HSV-2 primers (F:5´-CATGGGGCGTTTGACCTC-3' and R:5´-TACACAGTGATCGGGATGCT-3') with the amplicon size 249bp was used in this study, and PCR amplification was performed as previously described [16]. Briefly, 20μL of the reaction mixture containing 4μL of DNA template, 10μL of master mix, 4μL of double distilled water (H_2O), 1μL of both forward and reverse primers respectively, (20μL) of the reaction mixture was prepared for all 11 samples in the PCR tubes, followed by centrifugation at 5000rpm for 30 seconds and the tubes were covered. The reaction was performed in a programmable thermocycler (Agilent Technologies Surecycler 8800, Malaysia) for 40 cycles. Each amplification cycle consists of initial denaturation for 5 minutes at 94^{o}C, denaturation at 94^{o}C for 30 seconds, annealing for 30 seconds at 59.8^{o}C, initial extension at 72^{o}C for 30 seconds, and final extension at 72^{o}C for 5 minutes. The PCR products were visualized in1.5% Agarose gel stained with ethidium bromide. The gel was observed for the appropriate size DNA band under a UV trans-illuminator.

2.5 Data Analysis:

The data obtained were analyzed using the Statistical Package for SocialScience Statistical software (SPSS) version 20.0. A Chi-square test and bivariate analysis were performed to determine the association between variables. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

A total of 180 HIV-positive patients attending Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria, were recruited in this study; 136 (75.5%) were females, while 44(24.4%) were males. There was an overall HSV-

2 IgMseroprevalence of 11(6.1%) with 9(5.0%) among females and 2 (1.1%) among male participants (Fig. 1).

The age group of ≥ 45 years had the highest prevalence of 5(2.8%), while the least prevalence was among the age of 35–44 years with 2(1.1%). The findings also showed that females had the highest prevalence of 9(5.0%) and then males with 2(1.1%), although no significant association was found between age, gender, and the prevalence of HSV-2 ($P > 0.05$). This study obtained the highest prevalence among married participants of 7(3.9%), while single participants had the least prevalence of 1(0.6%). The difference was, however, not statistically significant ($P > 0.05$). The prevalence of HSV-2 concerning ethnicity; Hausa ethnic groups were found with the highest prevalence of 10(5.5%), then other tribes were 1(0.6%), and there was no statistical association between ethnicity and HSV-2 prevalence ($P > 0.05$). Similarly, the distribution of HSV-2 infection among the studied population based on residence showed that an overall prevalence of 11(6.1%) was observed among participants who lived in an urban area, and no significant association was obtained between residence and HSV-2 infection ($P > 0.05$). The highest prevalence was obtained 7(3.9%) among participants who had secondary education. In comparison, those with tertiary education had the least prevalence 4(2.2%), and there was no significant association ($P > 0.05$) between the educational level and the prevalence of HSV-2 ($P > 0.05$). The prevalence reported in this study concerning occupation was highest among Housewives 5(2.8%), followed by civil servants 4(2.2%), and the most minor was found among business people 2(1.1%). However, there was no significant statistical association between occupation and the prevalence of HSV-2 infection among the study population (Table 1).

Table 1
Seroprevalence of HSV-2 infection among people living with HIV concerning Socio-Demographic Characteristics (n = 180)

Demographical Data	HSV – Status		χ^2	Df	P – value
	No. Examined	Positive (%)			
Age (Years)					
15–24	64	4 (2.2)	0.480	3	0.100
25–34	5	0 (0.0)			
35–44	39	2 (1.1)			
≥ 45	72	5 (2.8)			
Gender					
Male	44	2 (1.1)	0.012	1	0.914
Female	136	9 (5.0)			
Marital Status					
Married	129	7 (3.9)	0.575	2	0.902
Single	9	1 (0.6)			
Widowed	42	3 (1.6)			
Ethnicity					
Hausa	151	10 (5.5)	0.552	3	0.795
Igbo	4	0 (0.0)			
Others	24	1 (0.6)			
Yoruba	1	0 (0.0)			
Residence					
Rural	31	0 (0.0)	1.172	**1**	0.280
Urban	149	11 (6.1)			
Educational level					
Primary	26	0 (0.0)	2.001	**2**	0.368
Secondary	101	7 (3.9)			
Tertiary	53	4 (2.2)			
Occupation					

Demographical Data	HSV – Status		χ^2	Df	P – value
	No. Examined	Positive (%)			
Age (Years)					
Business	90	2 (1.1)	7.102	5	0.213
Civil servant	39	4 (2.2)			
Farming	4	0 (0.0)			
Housewife	40	5 (2.8)			
Student	4	0 (0.0)			
Unemployed	3	0 (0.0)			

The seroprevalence of HSV-2 infection among people with HIV concerning associated risk factors was obtained. The history of STDs and the number of sex partners were not statistically significant with HSV-2 prevalence ($P > 0.05$). However, condom use was found statistically significantly associated with the prevalence of HSV-2 infection among the studied population ($P < 0.05$) (Table 2).

Table 2
Seroprevalence of HSV-2 infection among people living with HIV concerning risk factors (n = 180)

Risk Factors	HVS – 2 Status		OR	95CI	P value
	No. Examined	Positive (%)			
History of STDs					
Yes	66	5 (2.8)	1.475	0.432–5.036	0.748
No	114	6 (3.3)			
No. of Sex Partners					
0	1	0 (0.0)	-	-	0.243
1	146	7 (3.9)			
2	27	3 (1.7)			
3	6	1 (0.6)			
Sex Partners had other Partners					
Yes	92	7 (3.9)	1.689	0.477–5.984	0.538
No	87	4 (2.2)			
Use Condom					
Yes	87	1 (0.6)	9.355	1.173–74.600	0.026*
No	93	10 (5.6)			

Figure 2 shows the result of the Agarose gel image of the PCR for 1–11 samples, M is a 100bp ladder, NC is a negative control, Lanes 1–11 are the samples, 249bp is the amplicon size, samples with Lanes 1 2, 3, 7, and 11 were positives while 4,5,6, 8, 9 and 10 were negatives for PCR.

4. Discussion

To the best of our knowledge, this is the first study that determined the seroprevalence and molecular detection of HSV-2 in the study area. The overall seroprevalence of HSV-2 IgM (6.1%) reported in this study is lower than that of another study (37.3%) conducted in a tertiary setting of India among HIV-infected males with and without Genital Ulcer Disease (GUD using the ELISA method [6]. However, it is also far lower than those obtained by Nag *et al.*. [17], Hayatudeen *et al.*. [18], and Salman *et al.*. [19], among HIV/HSV-2 coinfected individuals (34.6%) in Eastern India, apparently healthy individuals (46.1%) in Nigeria, and Children under five years (20.37%) in Iraq, respectively, using the same ELISA method. Variations may be due to differences in the geographical locations and cultural/socio-demographic characteristics of the study participants and sample size.

On the other hand, the prevalence of HSV-2 infection in this present study is relatively high, particularly when compared with the similar study reported in a different part of Nigeria; Okonko *et al*. [20] said a prevalence of 2.8%. The higher prevalence reported in this study may be attributed to the higher risk of the study population exposed to HSV-2 infection, and biologically being HIV positive was associated with high HSV-2 seroprevalence [21]. The risk factors associated with HSV-2 disease identified in this study include the history of STDs, number of sex partners, use of condoms, and HIV status. This was in agreement with Hayatudeen et al.. [18], which also reported similar risk factors; sharing the exact route of transmission to HIV might also be the reason. However, another study conducted in the South-Eastern part of Nigeria obtained a higher prevalence of 85.7% among people living with HIV [22]. The variation may be due to differences in sexual behavior or socio-demographic characteristics of the study population [17]. On the other hand, the lower prevalence was also reported in similar studies [8, 14, 23]. This can be supported by the fact that HIV-1 infected individuals were more exposed to HSV-2 than other populations [22].

The higher prevalence of HSV-2/HIV co-infection among females than the males' counterparts reported in this study is consistent with similar studies [24, 25]. Moreover, reports from the United States [26] and Brazil [27] all reported females having a higher prevalence; this may be attributed to the differences in sample size and socio-demographic characteristics of the study population [25]. However, it may probably be due to the anatomical nature of female genitalia, which is more prone to infection, particularly STDs, or the possibility of women choosing sexual partners who are older than their age [28], and there is the higher transmission from men to women per exposure. Nevertheless, this has not attained the level of statistical association with HSV-2 infection. The distribution of HSV-2 disease was higher in the age group ≥ 45 years. This agrees with similar findings of similar studies [4, 29]. This may likely be as a result of the fact that HSV-2 infection persists for life, and seroprevalence increases with age throughout sexually active years [4]. The minor infected age group was 35–44 years. Agabi *et al*. [4] also made a similar observation. However, there was no statistically significant association between age and HSV-2 prevalence.

There was a prevalence rate of 1.6%, 0.6%, and 3.9% among widowed, single, and married participants, respectively. This can be explained by the fact that 30–50% of HIV infections in Sub-Saharan African countries can be attributed to HSV-2 disease [17]. It may likely also be due to active sexual life and probably extramarital affairs among married and widowed individuals since 95% of the individuals recruited in this study were married or widowed. A similar study reported a higher prevalence in singles compared to married ones [30]. Concerning ethnicity, the prevalence was higher among Hausa 5.5%, while other ethnic groups had a prevalence of 0.6% because the Hausa tribe was predominant in the study area. There was no statistical association between ethnicity and prevalence of HSV-2 infection ($P > 0.05$). About occupation, homemakers were recorded with the highest prevalence of 2.8%, followed by civil servants at 2.2%, and the least prevalence of 1.1% was reported among business participants. There was no statistically significant association between occupation and the prevalence of HSV-2 infection. This agrees with the report of Pennap and Oti [25]. Similarly, HSV-2 seroprevalence was higher among those

with secondary 3.9% education, followed by tertiary education 2.2%, and there was no statistical association between level of education and HSV-2 prevalence in the study population.

A statistically significant association was found between condom use and HSV-2 infection (P = 0.026). The prevalence was higher among those who did not use a condom for protection. This agrees with Kolawole et al.'s report [31], who found condom use was also statistically associated with HSV-2 prevalence. This might be attributed to culture and differences in the socio-demographic characteristics of the study population. Concerning the history of STDs and the number of sex partners, there was no statistically significant association with HSV-2 infection. The prevalence was higher among participants with one sex partner, 3.9%, followed by 1.7% for those with two sex partners and the least 0.6% for those with three sex partners. This is similar to the reports of Lupi [27]. Positive IgM ELISA and negative DNA results might be due to the persistence of the IgM antibodies for a long time after infection or cross-reactivity with other Alpha Herpesviruses. This agrees with the findings of Talaat *et al.*. [32], which reported positive IgM ELISA but negative PCR results in some individuals among pregnant women in Khartoum, Sudan.

Conclusion

This study confirmed the prevalence of HSV-2 infection among people living with HIV/AIDS in the study area. Not much attention were given because most of the individuals infected were unaware and asymptomatic. The prevalence of HSV-2 obtained in this study showed that condom use is not always practiced among the studied population. HSV-2 DNA has been detected in blood samples among some patients who were positive for HSV-2 IgM antibody. People living with HIV should be serologically tested for HSV-2 infection and counseled about the clinical and public health implications of the virus. The use of condoms for protection will also contribute immensely to reducing the burden of HSV-2 infection and HIV transmission.

Abbreviations

AKTH: Aminu Kano Teaching Hospital; BUK: Bayero University Kano; CDC: Centers for Disease Control and Prevention; DNA: Deoxyribonucleic Acid; ELISA: Enzyme-Linked Immunosorbent Assay; HIV/AIDS: Human immunodeficiency virus infection and acquired immunodeficiency syndrome; HRP: Horseradish Peroxidase; HSV-2: Herpes Simplex Virus Type-2; IgM: Immunoglobulin M; OR: Odds Ratio; PCR: Polymerase chain reaction; PLWHA: People living with HIV/AIDS; SPSS: Statistical Package for Social Science; STDs: Sexually Transmitted Diseases; STIs: Sexually transmitted infections.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Informed consent was obtained from all the patients recruited into the study. Ethical approval was obtained from the ethical committee of Aminu Kano Teaching Hospital, Kano State, Nigeria (NHREC/21/08/2008/AKTH/EC/2585). Furthermore, the study was approved by the Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, Bayero University Kano (BUK), Kano, Nigeria.

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the basis of this research. This research was conducted on humans are in accordance with the Helsinki Declaration of 1975, as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931).

CONSENT FOR PUBLICATION

Not applicable.

THE STANDARD FOR REPORTING:

STROBE guidelines and methodology were followed.

AVAILABILITY OF DATA AND MATERIALS

The data and materials are available on request.

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None

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none

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Figures

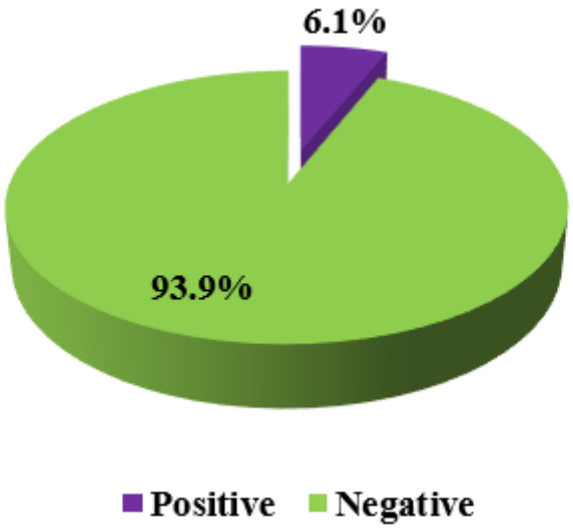

Figure 1

Seroprevalence of HSV-2 infection among people living with HIV/AIDS (n=180)

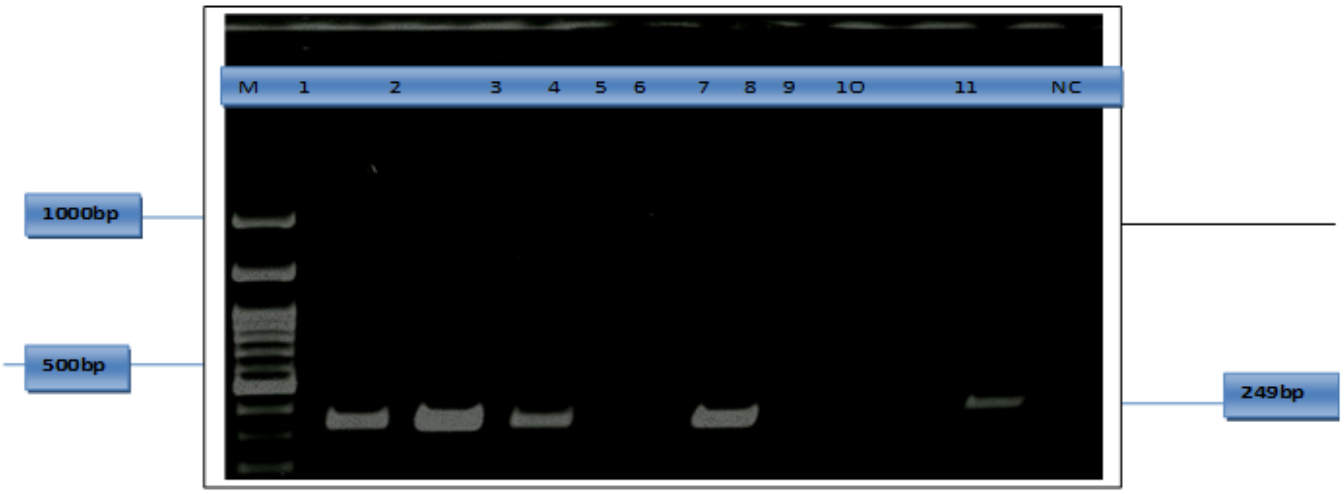

Figure 2

Results of Agarose gel Image of the PCR