

# Seroprevalence and Co-Infection of Human Immunodeficiency Virus (HIV) and Herpes Simplex Virus (HSV) Among Pregnant Women in Lokoja, North-Central Nigeria

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## Abstract

**Background:** Herpes simplex virus 1 (HSV-1) is normally associated with orofacial (orolabial) infections and encephalitis, whereas HSV-2 usually causes genital infections and can be transmitted from infected mothers to neonates. The evidence suggesting that HSV is facilitating the spread of the global human immunodeficiency virus (HIV) epidemic and the risk posed by these synergies to neonates in developing countries informed this study.

**Objectives:** To determine the seroprevalence and co-infection of HIV and HSV, as well as their associated risk factors, in Lokoja, Nigeria.

**Methods:** This was a hospital-based cross-sectional, prospective study, which was carried out among pregnant women attending the antenatal clinic at the federal medical centre in Lokoja, Nigeria. Sociodemographic characteristics and HIV-HSV status were determined by the use of a structured questionnaire and immunoassay kits, respectively. All data were analyzed using Stata statistical software (version 12), and the level of significance was determined to be  $P < 0.05$  using the chi-square test.

**Results:** Of the 250 pregnant women screened for HIV and HSV, 154 (61.6%) were in the 2nd trimester of gestation, and all of the co-infected respondents were in their 2nd trimester. Only six (2.4%) of the respondents tested positive for HIV, with all six (100%) showing positivity for HSV so the co-infection rate was six (2.4%). Co-infection was found to occur between the ages of 15 and 35 years, while higher age groups did not show any co-infection. Parity, level of education, and history of painful genital ulcers had no significant association with co-infection.

**Conclusions:** Advocacy and publicity to raise awareness of the potential public health impact of HSV and HIV co-infection in Nigeria, where anti-HSV testing is not generally performed in all populations, is therefore recommended.

**Keywords:** HIV, HSV, Co-infection, Pregnant Women, Lokoja

## 1. Background

Prior studies have focused on the possibility that cofactors may be important in the pathogenesis of acquired immunodeficiency syndrome (AIDS) (1, 2). Among the potential cofactors, herpes viruses are commonly mentioned because they are ubiquitous, have a particularly high prevalence in certain high-risk populations (3), and cause increased morbidity and mortality in patients with AIDS. Herpes simplex virus infection is said to be a significant factor in the increased risk of the acquisition and transmission of human immunodeficiency virus (HIV). In pregnant women, this may enhance the mother-to-child transmission (MTCT) of HIV. The reciprocal enhancement of HSV

and HIV viral replication in the presence of concurrent infection has also been described by several investigators (4, 5).

Herpes simplex viruses (HSV) are important human pathogens that cause diseases in different tissues and animal species. There are two antigenic types, HSV-1 and HSV-2, with HSV-1 being most commonly transmitted non-sexually and HSV-2 most usually being sexually transmitted (6). HSV-1 is usually associated with children, while HSV-2 is more prevalent in adults. Genital herpes disease is known to be of particular public health importance due to its morbidity, frequency of recurrence, and the rare but serious neonatal disease that may occur following intra-

partum transmission of HSV. It is highly prevalent in human populations in many parts of the world and it is the most common cause of genital ulcer disease (GUD) worldwide (7).

It is biologically plausible that GUD contributes to the high rates of 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 (8).

The epidemiological factors determining the persistent spread of this disease are similar to those of human immunodeficiency virus (HIV) infection, and both diseases have become mutually reinforcing epidemics (9). In some countries in the sub-Saharan region, where the vast majority of people living with HIV/AIDS reside, the prevalence of HSV in females of reproductive age has been said to range between 30 - 80% (10). This highlights the fact that the control of HIV and all other sexually transmitted infections (STIs) ought to be comprehensive rather than being focused on any particular agent. The risk of transmission and the severity of the neonatal disease have been reported to increase further if the woman is infected with HIV, more so if the newly infected pregnant woman did not have a prior HSV infection (11). HIV and HSV may also adversely affect the outcome of pregnancy, leading to spontaneous abortion, premature delivery, intrauterine growth restrictions, and low birth weight infants (12). To the best of our knowledge, there are no data currently available on the co-infection status of HIV and HSV among the populace in Lokoja, Nigeria. This study therefore investigates the seroprevalence of HIV and HSV co-infection in pregnant women in Lokoja, north-central Nigeria.

## 2. Objectives

The evidence that HSV is facilitating the spread of the global HIV epidemic and the risk that these synergies pose to neonates informed our study to determine the co-infection status of HIV and HSV among pregnant women attending the federal medical centre in Lokoja, north-central Nigeria, and identify the risk factors involved in the progression of the infection.

## 3. Methods

### 3.1. Study Area

This research was conducted at the federal medical centre in Lokoja, north-central Nigeria. Lokoja is the capital of Kogi state in Nigeria. Lokoja is located on 7°49'N

6°45'E/7.817°N 6.750°E and the climate is tropical in nature, characterized by tropical dry and wet seasons. The samples were collected from the federal medical centre, Lokoja, which is a 500-bed general government hospital that is also a training and referral center.

### 3.2. Study Population

The study population consisted of pregnant women who were newly enrolled at the antenatal clinic of the department of Obstetrics and Gynaecology at the Federal Medical Centre in Lokoja.

#### 3.2.1. Inclusion and Exclusion Criteria

A total of 300 women were sampled for this study, with only 250 of them meeting the inclusion criteria. The major inclusion criterion was pregnancy, while non-pregnant women were excluded from the study.

### 3.3. Sample Technique

The pregnant women were seen and a structured, closed-ended questionnaire was administered to participants after an informed consent form was completed. Consenting attendees were recruited and included in the survey, while those who were not interested were excluded. A specimen of each participant's blood was taken to be tested for HIV and HSV.

#### 3.3.1. Sample Size

The sample size was determined using Fisher's formula for a cross-sectional study:  $N = Z^2 pq/d^2$ , where N is the desired sample size, Z is the standard normal deviation (1.96), and P is the proportion of the target population estimated to have a particular characteristic (i.e., co-infection of HIV and HSV). Since there is no reasonable estimate, we use 50% (i.e., 0.5) for P.

### 3.4. Data and Blood Sample Collection

The questionnaire was given to the women after a verbal explanation was offered and informed consent was obtained. Some 5 mL of venous blood was collected aseptically from each study participant and then transferred into a vacutainer tube marked with a unique identifier.

### 3.5. Handling and Transportation of Samples

Separation of the serum was performed by the centrifugation of blood samples at 1,600 revolutions per minute (rpm) for 5 minutes using a bench-top centrifuge. The serum samples were collected into 1.8 mL cryo tubes (Nalge Nunc International, Rochester, New York, USA) and then stored at -20°C before being transported to the laboratory and stored at +4°C in a cold box with ice blocks.

### 3.6. HIV Assay

The serum samples were assayed in order to detect HIV. An assay for HIV antibodies was carried out on each specimen using a rapid immunoassay kit (Alere Determine™ HIV1/2, Chiba, Japan). All positive sample was retested using another immunoassay kit (Trinity Biotech Uni-Gold™ HIV, Wicklow, Ireland).

### 3.7. HSV Assay

The serum samples were assayed to detect HSV1/2 IgG antibodies using an EIA-ANTI-HSV-1/2-G-FAST ELISA KIT (Colchester, UK). This kit does not differentiate between HSV-1 and HSV-2.

### 3.8. Ethical Considerations

Approval for the study was obtained from the ethical committee of federal medical centre, Lokoja, on 17th October 2013. Informed consent was obtained from all patients and/or their parents/guardians, and the study was conducted at no cost to the subjects. Information from the patients or their parents was treated confidentially. The study was carried out between 1st November 2013 and 1st April 2014.

### 3.9. Data Analysis

All data were analyzed using Stata statistical software (version 12). Microsoft Excel 2010 for windows 7 was used for the tabulation of the results. The statistical level of significance was determined to be  $P < 0.05$  using the chi-square test.

### 3.10. Conflict of Interest

The authors report no conflicts of interest. This study was funded by contributions from each participating author.

## 4. Results

All respondents showed a 100% positivity for HSV 1/2 IgG antibodies, which cut across educational status, family type, age, motherhood status, and parity or gestation age. Condom use and history of STIs were extremely statistically significantly related to HSV infections ( $P = 0.0001$ ). Only 24.0% of respondents had 1-2 sexual partners, while 76.0% had more than two sexual partners in their lifetime ( $P > 0.999$ ) (Tables 1 and 2). Six of the pregnant women tested positive for HIV, thereby giving a seroprevalence of 2.4%. The risk factors for HIV did not show any statistical significance; however, it was noted that those respondents who were exposed to these factors had a higher prevalence

than those who were not (Table 3). There was a 2.4% co-infection rate of HIV and HSV among respondents. The rate of HIV and HSV co-infection in relation to the various demographic characteristics can be seen in Table 4. The history of GUD of those respondents who were co-infected with both viruses was found to be statistically significant (Table 5).

## 5. Discussion

This study shows a 100% HSV prevalence rate among pregnant women in Lokoja, north-central Nigeria. The high prevalence seen among pregnant women in this study is probably due to the fact the pregnancy itself reactivates the HSV virus (13). Physiological changes during pregnancy render the vaginal mucosa thinner and more easily damaged during sexual intercourse when compared to the non-pregnant vaginal mucosa. Thus, pregnant women may be more susceptible to HSV (14). Another reason for the 100% prevalence of HSV is the fact that HSV-1 and HSV-2 were not differentiated. The result from this study is consistent with the HSV prevalence level reported in a multi-center study conducted in four cities in sub-Saharan Africa: 90.0% in Cotonou, Benin republic; 96.6% in Benin, Nigeria; and 93.9% in Kisumu, Kenya (10, 15). However, this presents a much higher prevalence when compared to other prior study that reported a 24.4% HSV prevalence rate and a similar study among pregnant women in Norway (16, 17).

Condom use and history of STIs were extremely statistically significantly related to HSV infections ( $P = 0.0001$ ). The respondents who reported having three or more sexual partners in their lifetime (76%) were more likely to be infected with HSV when compared to respondents reporting fewer lifetime sexual partners (24%). The association between lifetime number of sexual partners and HSV is consistent with data from other studies and it is a measure of increased sexual activity (18).

An HIV seroprevalence rate of 2.4% was found in pregnant women in Lokoja, north-central Nigeria. This is similar to the rate found in a study among pregnant women in Okene, which reported a prevalence of 2.83% (19). The risks factors associated with the prevalence of HIV in this study were significant. Higher rates of the infection were found among respondents who were exposed to scarification, blood transfusion, razor blade incisions, and tattooing as compared to those who were not. The reason for this could be that adequate measures to prevent infection were not observed. This finding is in agreement with a study conducted in 2005, which reported an association of these factors with HIV infection (20-25).

The percentage of participants who were co-infected with HSV and HIV was found to be 2.4%. This is similar to the

**Table 1.** Prevalence of Herpes Simplex Virus in Relation to Condom Use and History of Sexually Transmitted Diseases Among Respondents<sup>a</sup>

Risk Factors	Yes	No	Total	P Value
Condom use	15 (6.0%)	235 (94.0%)	250 (100%)	0.0001
History of sti	50 (20.0%)	200 (80.0%)	250 (100%)	

<sup>a</sup>P < 0.05 = significant.**Table 2.** Prevalence of Herpes Simplex Virus in Relation to Lifetime History of Sexual Partner(s) Among Respondents<sup>a</sup>

	Frequency	Percentage	P Value
Number of sexual partner(s)			> 0.999
1-2	60	24.0	
More than 2	190	76.6	

<sup>a</sup>P < 0.05 = significant.**Table 3.** Prevalence of Human Immunodeficiency Virus in Relation to Associated Risk Factors<sup>a</sup>

Risk Factors	HIV Status		$\chi^2$ (P Value)
	No	Yes	
<b>Scarification</b>			0.278 (0.598)
Yes	180 (97.3%)	5 (2.7%)	
No	64 (98.5%)	1 (1.5%)	
<b>Blood transfusion</b>			0.177 (0.674)
Yes	237 (97.5%)	6 (2.5%)	
No	7 (100%)	0 (0%)	
<b>Tattooing</b>			0.025 (0.875)
Yes	243 (97.6%)	6 (2.4%)	
No	1 (100%)	0 (0%)	
<b>Razor blade incision</b>			0.283 (0.595)
Yes	233 (97.5%)	6 (2.5%)	
No	11 (100%)	0 (0%)	

<sup>a</sup>P < 0.05 = significant.

co-infection rate of 2.8% reported by a previous researcher (16). The co-infection rate of 2.4% obtained from this study is quite low when compared to the available information on the subject, especially among sex workers and other high-risk groups (26, 27). It is also lower than that found in a study conducted in Ibadan, Nigeria, which could be attributed to the low prevalence rate of HIV in the study area (28). Co-infection was found to occur in those between the ages of 15 and 35 years, while the higher age groups did not show any co-infection. Co-infection also occurred significantly among respondents who were educated (tertiary 50.0%, secondary 33.3%), which could be a result of the in-

fluence of education and public enlightenment/awareness concerning the disease among the people. It was observed that very few of the co-infected respondents knew the status of their partner, and only one acknowledged that her partner was positive for either of the two viruses. It was highly alarming that 83.3% of the co-infected respondents did not know their partner's status. This may be due to a lack of awareness of the disease (29). However, it was not a statistically significant finding ( $P = 0.056$ ). Co-infected respondents who had a history of GUD were more highly represented than those with no history. The relation between history of GUD and co-infection of HIV and HSV was statisti-

**Table 4.** Rate of HIV and HSV Co-infection Disaggregated by Demographic Characteristic

	Co-infection, n = 6		Non-Co-infection, n = 244		$\chi^2$ (P Value)
	Frequency	Percentage	Frequency	Percentage	
<b>Age, y</b>					6.816 (0.235)
15 - 19	1	16.7	4	1.6	
20 - 25	2	33.3	88	36.1	
26 - 30	2	33.3	102	41.8	
31 - 35	1	16.7	40	16.4	
36 - 40	0	0.0	4	1.6	
41 and above	0	0.0	3	1.2	
NA	0	0.0	3	1.2	
<b>Religion</b>					0.230 (0.631)
Christianity	2	33.3	104	42.6	
Islam	4	66.7	137	56.1	
NA	0	0.0	3	1.2	
<b>Marriage type</b>					0.546 (0.460)
Monogamous	5	83.3	210	86.1	
Polygamous	1	16.7	23	9.4	
NA	0	0.0	11	4.5	
<b>employment status</b>					1.651 (0.648)
Employed	2	33.3	60	24.6	
Self-employed	1	16.7	95	38.9	
Unemployed	2	33.3	66	27.0	
Student	1	16.7	18	7.4	
NA	0	0.0	5	2.0	
<b>Level of education</b>					1.169 (0.76)
No education	1	16.7	21	8.6	
Primary	0	0.0	28	11.5	
Secondary	2	33.3	86	35.2	
Tertiary	3	50.0	106	43.4	
NA	0	0.0	3	1.2	
<b>Parity status</b>					1.883 (0.170)
Yes	2	33.3	149	61.1	
No	4	66.7	95	38.9	
<b>No. of children</b>					3.769 (0.152)
None	4	66.7	95	38.9	
One	2	33.3	58	23.8	
Two	0	0.0	57	23.4	
Three	0	0.0	13	5.3	
Four	0	0.0	14	5.7	
Five	0	0.0	7	2.9	
<b>Trimester</b>					3.598 (0.609)
One	0	0.0	43	17.62	
Two	6	100.0	149	61.07	
Three	0	0.0	52	21.31	

cally significant ( $P = 0.007$ ), and this is in accordance with the findings of some previous studies (30, 31). In this study, it was clearly established that all the subjects with an HIV infection also had an HSV infection. This is not surprising, as it has been previously shown that the presence of the HSV infection plays a critical role in the acquisition of the HIV infection (32).

It is worth noting that there was a consistently high prevalence of HSV infection irrespective of educational status, family type, age, motherhood status, and parity or gestation age. This could be attributed to re-infection due to the continuous transmission of the virus in society, leading to a higher production of the corresponding antibody, which is in accordance with the situation described by Obi-

**Table 5.** Risk Factors for Human Immunodeficiency Virus and Herpes Simplex Virus Co-Infection in Respondents

Risk Factors	Co-Infection, N = 6	Non-Co-Infection, N = 244	$\chi^2$ (P Value)
	Frequency	Frequency	
<b>Infected partners</b>			5.780 (0.056)
Yes	1 (16.7%)	10 (4.1%)	
No	0 (0%)	85 (34.8%)	
Don't know	5 (83.3%)	149 (61.1%)	
<b>GUD</b>			7.231 (0.007)
Yes	5 (83.3%)	17 (7.0%)	
No	1 (16.7%)	227 (93.1%)	

jimi et al. (33) and Agabi et al. (34).

The results of this study highlight the potential public health impact of HSV in Nigeria, where anti-HSV testing is not generally performed in all populations, especially considering the risk of neonatal transmission and the attendant complications at birth, as well as the synergy between HIV and HSV transmission (35). There was no strong association between HSV and HIV infection in the area under study. There is thus a need for further studies in this part of the country to validate the link between the two infections. A major novelty of this study is that, to the best of our knowledge, no other study of this type has been conducted in the study area. The present study therefore provides baseline information on the co-infection of HIV and HSV as well as their associated risk factors.

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### Footnote

**Authors' Contribution:** All authors of this research paper have directly participated in the planning, execution, or analysis of this study.

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