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# PREVALENCE AND DETERMINANTS OF HERPES SIMPLEX VIRUS 2: A RURAL-URBAN COMPARISON AMONG HIV-INFECTED AND UNINFECTED PREGNANT WOMEN IN CROSS RIVER STATE, NIGERIA

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***Abstract:*** The prevention of neonatal herpes is an important intervention in the fight against herpes simplex virus infection. It can only be of benefit if the HSV status of the mother is known. In addition, HSV infection is a biological cofactor for HIV infection, a disease of pandemic proportion. Screening pregnant women for HSV infection would help identify at risk women and allow for interventions to prevent neonatal infection. To compare the seroprevalence of HSV type 2 among HIV infected and HIV uninfected pregnant women. Serum samples from 300 pregnant women (150 HIV infected and 150 HIV uninfected) were tested for HSV-2 IgG antibodies. Participants who were women below 36 weeks' gestation received voluntary counseling after which their HIV and HSV-2 status were determined using a commercial kit (CALBIOTECH HSV-2 IgG ELISA). The seroprevalence of HSV type 2 as well as prevalence of HSV type 2 and HIV co-infection was determined. A comparison was made of the seroprevalence of HSV type 2 among HIV infected and HIV uninfected clients. Rural-urban comparison was also made of the prevalence of HSV type 2. Data obtained were analyzed using SPSS. Multivariate logistic regression was used to identify independent predictors of HSV type 2 infections. Prevalence of HSV-2 among HIV infected pregnant women was 83.3%, HIV uninfected pregnant women was 80.7%, rural dwellers 74.4% and urban dwellers 87.4%. Factors associated with HSV-2 seropositivity were knowledge about HSV-2 ($X^2$=5.426; p=0.020), respondent location ($X^2$=13.102; p=0.001), and spousal age (FET;9.933, p=0.047). Spousal age was an independent predictor of HVS type 2 seropositivity using a multivariate binary logistic regression.

The prevalence of HSV-2 is relatively high among pregnant women in Cross River state, Nigeria. There is need to develop strategies aimed at reducing HSV-2 infection among pregnant women

***Index Terms* -** Herpes simplex virus, Human immunodeficiency virus, Sero prevalence, Enzyme Linked Immunosorbent assay.

## INTRODUCTION

Genital herpes simplex virus (HSV) infection is one of the most common sexually transmitted diseases with an estimated 50 million adolescents and adults currently affected in the United State of America.[1] It is now recognized as the most common cause of genital ulcer disease worldwide.[2] As most cases of HSV are transmitted by persons who are asymptomatic or unaware of their disease, this has become a major public health concern. Although most women are unaware of their infection, about one in five has serological evidence for HSV-2 infection.[3,4] HSV-2 is recovered almost exclusively from the genital tract and is usually transmitted by sexual contact. Genital herpes may be caused by either HSV-1 or HSV-2 but, globally, the large majority (90%) of cases is caused by HSV-2.[2] The acquisition of genital herpes during pregnancy has been associated with spontaneous miscarriage, premature labour and congenital and neonatal herpes. Cengiz et al[5], in their study on 73 mothers with various obstetric problems like abortion, still birth, premature birth and intrauterine developmental retardation, reported HSV-2 IgG positivity in 65 (89.1%) and HSV-2 IgM positivity in 6 (8.2%). Unrecognized viral shedding from the mother's genital tract may have occurred at the time of delivery in as many as 70 percent of cases of neonatal herpes.[6,7,8] Neonate with disseminated herpes simplex infection has a mortality rate of nearly 30 percent even with acyclovir treatment.[9]

HSV-2 is a lifelong and incurable infection that can cause recurrent and painful genital sores and can make those infected with the virus two-to-three times more likely to acquire HIV, the virus that causes AIDS. As HSV-2 is almost always sexually transmitted, it thus serves as a good marker of sexual behavior in the population.[10,11] Seroprevalence studies show wide variations in infection rates by geographical location. Highest prevalence of HSV-2 has been found in some parts of Africa, America and the lowest in Asia.[12] Globally about 535.5 million were infected with HSV-2, with an overall prevalence of 16.2% in 2003.[13] The prevalence in South-Asia in 2003 was estimated to be 29.4% and 33.4% among males and females in the group of 15-49 years respectively.[13] Recent study from India shows HSV-2 prevalence among general population to be around 10%.[15]

HSV-2 is more common in Sub-Saharan Africa than in Europe or the North America. Up to 82% of women and 53% of men in Sub-Saharan Africa are seropositive for HSV-2. These are the highest levels of HSV-2 infection in the world, although exact levels vary from country to country in this continent.[16] In most African countries, HSV-2 prevalence increases with age. However, age-associated decrease in HSV-2 seroprevalence has been observed for women in Uganda and Zambia, and in men in Ethiopia, Benin, and Uganda.[17] HSV-2 is gaining special attention as a significant risk factor for acquisition of human immunodeficiency virus type-1 (HIV-1). HSV-2 is also present in 30 to 70% of those in Europe and 50 to 90% of those in Africa among patients with HIV infection.[18] HSV-2 increases the risk of HIV-1 transmission by more than two fold and HIV transmission on a per-sexual act basis by up to fivefold and may account for 40-60% of new HIV infections in high HSV-2 prevalent populations.[19-26] It is assumed that infection with HSV-2 disrupts the genital mucosa and provides a portal of entry for HIV, leading to increase susceptibility of HIV in HIV-negative persons. Also, HSV-2 is thought to enhance HIV acquisition due to dense inflammatory infiltrates of CD4/CD8/dendritic cells in the genital tract associated with HSV-2 shedding.[27] Moreover, in HIV-positive persons, infection with HSV-2 accelerates replication and genital shedding of the virus, thus such individuals are more likely to transmit HIV.[28,29] Also, other meta-analysis data from general population among women showed that in prevalent HSV-2 infection, HIV acquisition increased by over a three- fold.[30] Nagot et al. highlighted the association of HSV-2 with significantly higher viral load of HIV-1 in plasma and in genital secretions in women with sexually acquired HIV-1.[29]

The most severe consequence of herpes simplex virus type 2 (HSV-2) infections is related to its transmission from the mother to the newborn during delivery.[33] In the adult patient, genital herpes could have significant psychosocial repercussions. There is little information on the prevalence of HSV-2 in Nigeria despite the high burden of the disease in Sub-Saharan Africa. The first documented isolation of HSV-2 in Nigeria was done in Ibadan.[34] A sero-survey of *Haemophilis ducreyi,* syphilis, and herpes simplex virus type-2 and their association with HIV among female sex workers in Lagos, Nigeria reported a seroprevalence of 59% for HSV-2.[35] In another seroprevalence study involving 164 patients attending sexually transmitted infections clinic at Jos University Teaching Hospital, a prevalence of 87% was obtained.[36]

This result is consistent with HSV-2 prevalence reported in a multi-centre study in four sub-Saharan African cities: 90% in Cotonu, Benin Republic; 84.1% in Yaoundé, Cameroun; 93.9% in Kisumu, Kenya; and 87.7% in Ndola, Zambia, as of June 1997 to March 1998.[16]

A more recent study done among 180 pregnant women attending antenatal clinic at Braithwaite Memorial Specialist Hospital (BMSH) in Port Harcourt, Nigeria reported a seroprevalence of 99.4% for HSV 1&2 IgG antibody.[37] This finding has direct clinical implications, suggesting that elimination of HSV-2 viral shedding and genital tract inflammation can reduce the transmission and acquisition of HIV infection. HSV-2 as a biological cofactor in HIV acquisition and transmission have contributed substantially to HIV infections particularly by facilitating HIV spread among the low-risk population with stable long-term sexual partnerships.[31]

## 1.1 PATIENTS AND METHODS

This study is a comparative cross sectional hospital based study. The study was conducted in four centers in Cross River State, Nigeria. These centers include; University of Calabar Teaching Hospital, General Hospital Calabar, General Hospital Akamkpa and Cottage Hospital Uyanga Akamkpa. The first two centers are located in Calabar municipality while the last two are located in Akamkpa Local Government Area respectively. Both local government areas are located in Southern Senatorial District of Cross River State. University of Calabar Teaching Hospital is the only tertiary healthcare facility in Cross River state, South-south geographical region of Nigeria. It provides healthcare services to the over 3.3million people in the state and its environs. It offers antenatal care and PMTCT services to all pregnant women. HIV counseling and testing is offered to all antenatal clients on the booking clinic using the opt out approach. The HIV screening uptake is about 96%. Posttest counseling is offered to all tested pregnant women irrespective of their test result. All the pregnant women who tested positive to HIV are commenced on antiretroviral medications. The HIV prevalence rate is 3%.

### 1.1.2 Laboratory Procedures

The collected samples were allowed to clot and sera separated by centrifugation for 15 minutes at room temperature. The resultant sera were extracted into labeled Eppendorf tubes. Sera were stored at temperature of -20 degree Celsius until use.

### 1.1.3 Serology Principle

This assay measures IgG to herpes simplex virus-2. It is based on a non-competitive or sandwich enzyme linked immunoassay technique. Samples were tested for HSV-2 IgG antibody using the CALBIOTECH HSV-2 IgG ELISA serology testing as per the manufacturer instructions (Calbiotech, Inc. 1935 Cordell Ct., El Cajon, CA 9202 U.S.A. The microwells are coated with purified antigen specific for IgG antibody to herpes simplex virus 2. Herpes simplex virus 2 IgG antibodies in callibraor, control and samples bind to the antigen. The unbound IgG antibodies are washed off. Enzyme conjugate is added. The enzyme conjugate binds to Herpes simplex virus 2 IgG immobilized by the antigen to form a sandwich complex. The unbound enzymes conjugate are washed off. Enzyme substrate is added with resultant colour development. The intensity of the colour is proportional to the concentration of Herpes simplex virus 2 IgG antibody. The absorbance of Herpes simplex virus 2 IgG in the samples are read at a wavelength of 450nm.

### 1.2 Materials and Equipment Required

i. Microwell plates (96wells)

ii. Polypropylene tubes

iii. Cardboard sealers

iv. Calibrated prension micropipettes (10 – 1000ul)

v. Absorbent papers

vi. Automatic microwell plate washer

vii. Microwell reader

viii. Deionised water

### 1.2.1 Reagents Required

i. Serum samples

ii. Sample diluents

iii. HSV – IgG calibrator

iv. Quality control sera

v. Enzyme conjugate solution

vi. Substrate solution

vii. Wash solution

viii. Stop solution

### 1.2.2 Assay Procedure

i. All samples and reagents were brought to room temperature

ii. The desired number of wells were secured in the microwell holder

iii. Samples were diluted by the addition of 10ul of the sample to 200ul of sample diluents

iv. 100ul of sample diluents was added to the first microwell for blanking purpose

v. 100ul of calibrator, controls and diluted samples were added to the appropriate wells

vi. The microwells were covered with adhesive tape and incubated for 20 minutes at room temperature

vii. The microwells were washed three times with diluted wash solution (300ul/well)

viii. The wash solution was removed from the wells by inverting the plate

ix. The microwells were then dried by inversion on absorbent paper

x. 100ul of enzyme conjugate was added to each well and incubated for 20 minutes

xi. The microwells were washed three times with dilute wash buffer

xii. The wash solution was removed from the wells by inverting on a plate and dried by inversion on absorbent paper

xiii. 100ul of substrate solution was dispensed into the microwells

xiv. The microwells were incubated for 10minutes at room temperature

xv. The colour development was read at a wavelength of 450nm with their respective absorbance values recorded

### 1.3 Calculation of Results

i. The absorbance values of the calibrator, controls and samples were recorded

ii. The calibrator factor on the calibrator bottle was recorded

iii. The cut off value (cov) was determined using the following formula.

Cut off value (cov) = calibrator optical density (absorbance) × calibrator factor (cf)

iv. The antibody index of each sample was determined by dividing the optical density (absorbance) value of each sample by the cut off value Antibody index of each sample

v. The antibody index (A1) value of each sample was used to determine samples with detectable antibody to Herpes simplex virus – 2 (HSV – 2) IgG using defined criteria as follows:

- ✓ Antibody index < 0.9: No detectable antibody to HSV – 2 IgG
- ✓ Antibody index 0.9 – 1.1: Borderline positive. Follow up testing indicated if clinically indicated
- ✓ Antibody index > 1.1: Detectable antibody to HSV – 2 IgG

Quality control and test validation was included into the test protocols. The other portion of the sera is tested for HIV using the Determined Rapid Test Kit (Alere Determine$^{TM}$ HIV-1/2 SET, Alere Medical Co., Ltd. 357 Matsuhidai, Matsudo-shi, Chiba, 270-2214, Japan, www.alere.com). 50µl of sera is applied to the test strip. Once it is absorbed, 2 drops of buffer were applied to the same spot. The result was read after 20 minutes. A control (red) line, which indicates assay validity, would be evident. The control line appearing alone, gives a negative result but when combined with a 2$^{nd}$ red line (test line), the result is positive. The Rapid test kits contain HIV 1/2 recombinant antigen and synthetic peptide coated test cards for in vitro diagnostic use. The above kits were stored at temperature between 2 and 30$^{0}$C. They can be used with whole blood, plasma or serum. Before use, the kit was inspected for damages and the expiration date noted.

## 2.0 RESULTS

### 2.1. Socio-demographic characteristics of women

A total of 300 women participated in the study out of which 150(50.0%) were HIV infected while 150(50.0%) were HIV uninfected. Those in the 26-30 years' age group accounted for the highest overall proportion of 98(32.7%) study participants and this was relatively higher in the HIV uninfected group, 56(37.3%) compared with the infected group, 42(28.0%). The difference in age between the two comparative groups was statistically significant (p<0.001). However, the overall mean age of the study participants was 30.16 ± 5.56, higher among HIV infected (31.77 ± 5.75) than the HIV uninfected group (28.54 ± 4.87) and the difference was statistically significant (p<0.001). Similarly, the difference in marital status (p<0.001) as well as in level of education (p<0.001) were statistically significant. On the other hand, there were no statistically significant difference in occupation (p=0.137) as well as in tribe (p=0.480). The difference in religion distribution between the HIV infected and the HIV uninfected was significant (p<0.001), Table 1).

**2.1.1 Tables**

**Table 1: Socio-demographic characteristics of pregnant women (N=300)**

| Variable | HIV infected n=150 | HIV uninfected n=150 | Total N=300 | Fisher's Exact Test | p-value |
|---|---|---|---|---|---|
| **Age group/years** | | | | | |
| 20 and below | 4(2.7) | 9(6.0) | 13(4.3) | 26.877 | <0.001* |
| 21-25 | 18(12.0) | 36(24.0) | 54(18.0) | | |
| 26-30 | 42(28.0) | 56(37.3) | 98(32.7) | | |
| 31-35 | 48(32.0) | 36(24.0) | 84(28.0) | | |
| 36-40 | 30(20.0) | 13(8.7) | 43(14.3) | | |
| 41 and above | 8(5.3) | 0(0.0) | 8(2.7) | | |
| Mean age ± SD | 33.7±5.6 | 31.6±4.3 | 32.7±5.0 | T-test(1.725) | =0.135 |
| **Marital status** | | | | | |
| Married | 121(80.7) | 144(96.0) | 265(88.3) | 17.870 | <0.001* |
| Single | 13(8.7) | 4(2.7) | 17(5.7) | | |
| Separated | 4(2.7) | 0(0.0) | 4(1.3) | | |
| Divorced | 3(2.0) | 1(0.7) | 4(1.3) | | |
| Cohabiting | 9(6.0) | 1(0.7) | 10(3.3) | | |
| **Highest education** | | | | | |
| None | 3(2.0) | 0(0.0) | 3(1.0) | 16.172 | <0.001* |
| Primary | 16(10.7) | 6(4.0) | 22(7.3) | | |
| Secondary | 76(50.7) | 58(38.7) | 134(44.7) | | |
| Tertiary | 55(36.7) | 86(57.3) | 141(47.0) | | |
| **Occupation** | | | | | |
| Civil servant | 15(10.0) | 20(13.3) | 35(11.7) | 12.721 | 0.133 |
| Public servant | 11(7.3) | 16(10.7) | 27(9.0) | | |

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|---|---|---|---|---|---|
| Company staff | 7(4.7) | 6(4.0) | 13(4.3) | | |
| Businesswoman | 65(43.3) | 42(28.0) | 107(35.7) | | |
| Apprentice | 0(0.0) | 3(2.0) | 3(1.0) | | |
| Student | 9(6.0) | 12(8.0) | 21(7.0) | | |
| Housewife | 22(14.7) | 29(19.3) | 51(17.0) | | |
| Unemployed | 6(4.0) | 11(7.3) | 17(5.7) | | |
| Others | 15(10.0) | 11(7.3) | 26(8.7) | | |
| **Tribe** | | | | | |
| Efik | 37(24.7) | 41(27.3) | 78(26.0) | 4.565 | 0.480 |
| Ejagham | 67(44.7) | 59(39.3) | 126(42.0) | | |
| Ibibio/Anang | 29(19.3) | 23(15.3) | 52(17.3) | | |
| Ibo | 14(9.3) | 21(14.0) | 35(11.7) | | |
| Hausa | 2(1.3) | 2(1.3) | 4(1.3) | | |
| Yoruba | 1(0.7) | 4(2.7) | 5(1.7) | | |

*=Statistically significant

Table 2 shows obstetrics characteristics of study participants. A relatively higher proportion of the HIV uninfected women compared with the HIV infected women were nulliparous (35.3% versus 16.7%) or primiparous (26.0% versus 20.7%). However, among those in the multiparous (58.7% versus 38.7%) or grand multiparous (4.0% versus 0.0%) subgroups the proportions were more in the HIV infected compared with the uninfected. The difference by parity was statistically significant ($p<0.001$). Mean parity was higher in the HIV infected (2.26 ± 1.84) compared with HIV uninfected (1.28 ± 1.22) and this difference was significant ($p<0.001$). Although gestational age category did not show any significant difference, their difference in mean did ($p=0.031$), being significantly higher in the HIV uninfected group (25.97 ± 6.59) compared with the HIV infected group (24.27 ± 6.97). A higher proportion in the HIV uninfected group had adequate ANC attendance (84.0% versus 49.3%) and previous abortion (51.3% versus 48.0%) which showed significant difference ($p<0.05$) whereas, more from the HIV infected women had previous still birth (13.3% versus 10.0%), previous caesarean section (20.7% versus 16.0%), previous episiotomy (22.7% versus 14.7%), previous obstetrics history (6.0% versus 3.3%) which did not show any significant difference statistically ($p>0.05$).

**Table 2: Obstetrics characteristics of study participants (N=300)**

| Variable | HIV infected n=150 | HIV uninfected n=150 | Total N=300 | Fisher's Exact test | p-value |
|---|---|---|---|---|---|
| **Parity** | | | | | |
| Nulliparous (0) | 25(16.7) | 53(35.3) | 78(26.0) | 23.301 | <0.001* |
| Primiparous (1) | 31(20.7) | 39(26.0) | 70(23.3) | | |

| | | | | | |
|---|---|---|---|---|---|
| Multiparous (2-5) | 88(58.7) | 58(38.7) | 146(48.7) | | |
| Grand multiparous (>5) | 6(4.0) | 0(0.0) | 6(2.0) | | |
| Mean parity ± SD | 2.26 ± 1.84 | 1.28 ± 1.22 | 1.77 ± 1.63 | T-test; 5.426 | |
| **GA/weeks** | | | | | |
| 1-13 | 9(6.0) | 2(1.3) | 11(3.7) | 5.249 | 0.072 |
| 14-26 | 78(52.0) | 75(50.0) | 153(51.0) | | |
| >26 | 63(42.0) | 73(48.7) | 136(45.0) | | |
| Mean GA ± SD | 24.27 ± 6.92 | 25.97 ± 6.59 | 25.12 ± 6.83 | T-test (2.161) | 0.031* |
| **Adequate ANC** | | | | | |
| Yes | 74(49.3) | 136(84.0) | 200(66.7) | 42.225 | <0.001* |
| No | 76(50.7) | 24(16.0) | 100(33.3) | | |
| **Any previous abortion?** | | | | | |
| Yes | 72(48.0) | 77(51.3) | 149(49.7) | 37.762 | <0.001* |
| No | 69(46.0) | 30(20.0) | 99(33.0) | | |

Table 3 represents association between obstetrics characteristics and HSV type 2 seropositivity among pregnant women. Multiparous with a proportion of 84.9%, follow by grand multiparous women with a proportion of 83.3% who had HSV type 2, implying that increasing parity increases the likelihood of HSV type 2 seropositivity although the relationship was not statistically significant (p=0.507). Again, there was no statistically significant relationship between HSV type 2 seropositivity and previous abortion (p=0.055), adequate ANC (p=0.27), still birth (p=0.743), caesarean section (p=0.930), episiotomy (p=0.770), obstetrics hospitalization (p=0.457) and other obstetrics history (p=1.000).

**Table 3: Association between obstetrics characteristics and HSV type 2 seropositivity among pregnant women (N=300)**

| **Variable** | **HSV type 2 positive (n=246)** | **HSV type 2 negative (n=54)** | **Total (N=300)** | **Fisher's Exact Test** | **p-value** |
|---|---|---|---|---|---|
| **Parity** | | | | | |
| Nulliparous (0) | 63(80.8) | 15(19.2) | 78(100.0) | 2.246 | 0.507 |
| Primiparous (1) | 54(77.1) | 16(22.9) | 70(100.0) | | |
| Multiparous (2-5) | 124(84.9) | 22(15.1) | 146(100.0) | | |

| | | | | | |
|---|---|---|---|---|---|
| Grand multiparous (>5) | 5(83.3) | 1(16.7) | 6(100.0) | | |
| Mean parity SD | | | | | |
| **Adequate ANC?** | | | | | |
| Yes | 166(82.5) | 34(17.5) | 194(100.0) | 1.029 | 0.627 |
| No | 80(80.0) | 20(20.0) | 100(100.0) | | |
| **Had previous abortion?** | | | | | |
| Yes | 118(79.2) | 31(20.8) | 149(100.0) | 6.706 | 0.055 |
| No | 89(89.9) | 10(10.1) | 99(100.0) | | |
| No response | 39(75.0) | 10(25.0) | 52(100.0) | | |
| **Had previous stillbirth?** | | | | | |
| Yes | 28(80.0) | 7(20.0) | 35(100.0) | 0.107 | 0.743 |
| No | 218(82.3) | 47(17.7) | 265(100.0) | | |
| **Previous C/S?** | | | | | |
| Yes | 46(83.3) | 9(16.7) | 55(100.0) | 1.021 | 0.930 |
| No | 200(81.6) | 45(18.4) | 245(100.0) | | |
| **Had previous episiotomy?** | | | | | |
| Yes | 45(80.0) | 11(20.0) | 56(100.0) | 0.722 | 0.770 |
| No | 201(82.4) | 43(17.6) | 244(100.0) | | |
| **Previous obstetrics hospitalization?** | | | | | |
| Yes | 41(77.4) | 12(22.6) | 53(100.0) | 1.491 | 0.457 |
| No | 205(82.9) | 42(17.1) | 247(100.0) | | |
| Other obstetrics history? | | | | | |
| Yes | 12(85.7) | 2(14.3) | 14(100.0) | - | 1.000 |
| No | 234(81.8) | 52(18.2) | 286(100.0) | | |

## DISCUSSION

The study showed HSV 2 prevalence of 80.7% among HIV uninfected pregnant women and 83.3% among HIV Infected pregnant women. This is generally high and consistent with high HSV-2 prevalence reported in a multi-centre study conducted in four Sub-Saharan African cities; 90% in Cotonu, Benin Republic, 84.1% in Yaoundé, Cameroun, 93.9% in Kisumu, Kenya and

87.7% in Ndola, Zambia.[16] However, this prevalence is higher when compared to an earlier study done among pregnant women in Sub-Saharan Africa (40%),[26] Sweden (10.4)[43] and China (10.8). The higher prevalence among HIV infected women albeit not significant is similar to other studies done in the sub-region that reported high prevalence among HIV positive individuals.[18] Multicenter study done in Northeast India also confirmed high prevalence of HSV-2 among population with highest HIV prevalence.

The study also showed a higher prevalence among urban (87.4%) and rural (74.4%) dwellers, though there is a significant relation between location of the parturient and the risk of acquiring HSV-2, with urban dwellers more likely to acquire HSV-2 than those living in rural areas. However, the high prevalence among the two arms may be explained by human interaction between the study centers. The study centers are not far apart. This study reported a high prevalence of HSV-2 among rural dwellers (74.4%) compared to studies done in rural Ethiopia (32.1%) and rural Tanzania.[48] The statistically significant higher prevalence among the urban dwellers may be explained by the fact that men in urban locations are more likely to be involved in risky sexual behavior standing a higher risk of infecting their wives with HSV-2 virus. This study is consistent with a study done in Haiti that reported a significantly high prevalence among urban dwellers (57.6%) compared to rural dwellers (42.4%). The high prevalence of HSV-2 among the study population may be due to poor awareness of HSV-2 infection and its transmission among the general population.

The study also showed that more than two third (86.0%) of the study population are unaware of HSV-2 infection. Of the few who have heard about HSV-2, there is a statistical significant(p=0.02) difference among the study groups, with two times more HIV-uninfected pregnant women likely to have heard about HSV-2 infection than HSV-infected (18.7% versus 9.3%). This also reflected on the knowledge about the mode of transmission of HSV-2 as more HIV-uninfected women are aware of the methods of HSV-2 transmission. The study did not show consistency in the HSV-2 related symptoms and HIV status. More HIV infected pregnant women admitted to have had vaginal discharge, vulval itching and genital blisters whereas more respondents from the HIV-uninfected group had genital ulcers, painful urination and feeling of unwell.

The significance of these findings may be hampered by the fact that there are lots of other organisms that could cause same symptoms. For example, candida albicans, bacterial vaginosis and trichomonas vaginalis will cause vaginal discharge, vulval itching and dysuria whereas organisms such as herpes simplex virus, varicella zoster, syphilis, granuloma inguinale can cause genital blisters and ulcers. Genital blisters and ulcers can also result from none sexually transmitted conditions such as allergies, trauma, scabies, erythema multiforme, pediculosis and furuncles. Though marital status was reported to be significant in determining the HIV status of the women (p<0.001), there is no statistically significant relationship between HSV-2 and marital status. The study showed that all pregnant women who were separated were seropositive. Seropositivity was also high among single pregnant women. Studies done in Port Harcourt and Jos, Nigeria, reported high prevalence of HSV-2 among single women.[36,37]

However, this finding contradicts an Iranian study that reported high prevalence of HSV-2 among married women. This may be explained by the fact that single and separated women are more likely to be involved in multiple sexual relationships and high-risk sexual behavior. The study did not show any statistically significant relationship between HSV-2 seropositivity and sociodemographic characteristics such as level of education, occupation, tribe and religion. Also, the prevalence of HIV in this study is not affected by occupation and tribe and religion. However, education has a bearing on the prevalence of HIV in this study. There is a statistical level of significance between spousal age and HSV-2 seropositivity among the study participants (p=0.047).

Cross-generational sex is a common finding in our environment as more young girls marry older men for economic reasons, though the study did not compare women age at first sexual contact with the age of their partners and risk of HSV-2/HIV acquisition. The study also showed a statistical level of significance between spousal educational status (p<0.001), occupation (p<0.035) and HIV acquisition; however, these variables had no significant bearing on HSV-2 seroprevalence. It is difficult to make comparison as no study in our setting has compared spousal level of education, occupation and HSV-2 seroprevalence among pregnant women. Low levels of education, unemployment are features of low socioeconomic status and are associated with poor health seeking behavior. Studies done in rural Mysore, Taluk, India, reported that pregnant women whose partner ever traveled had 2.68 times the odds of being HSV-2 positive compared with women whose partners never traveled (95% CI 1.14-6.34). An Australian study showed low prevalence of HSV-2 among people of low socioeconomic status compared to higher socioeconomic status.

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Higher parity increased the likelihood of HSV-2 seropositivity though not statistically significant (p=0.507). Seropositivity was highest among multiparous pregnant women (84.9%) followed by grand multiparous pregnant women (83.3%). Other obstetrics characteristics such as previous abortion, adequate ANC, still birth, caesarean section, episiotomy and obstetrics hospitalization did not significantly affect HSV-2 seropositivity in this study. On the other hand, mean parity was higher in the HIV infected group compared to HIV uninfected and this difference was significant (p<0.001). This study adds to the limited information on HSV-2 prevalence in Nigeria, in spite of its limitations. The test results obtained cannot discriminate between HSV-1 and HSV-2 infection due to high cross reactivity between the two viruses. This could lead to high false positive result. This is a common finding among African population. Moreover, confirmatory test was not carried out on borderline positive values to ascertain their true serostatus. Since the production of IgG antibodies following HSV-2 infection takes about 3 to 4 weeks, the

study may be unable to identify infected pregnant women who are yet to produce IgG antibodies to HSV-2. Additionally, I may have introduced selection bias into the study by using convenient sampling technique for recruiting HIV positive pregnant women into the study. I accounted for unequal probabilities of selection for the HIV-positive women and randomly selected HIV-negative women using sampling weight. This study has highlighted the high prevalence rate of HSV-2 infection, the low level of awareness about HSV-2 infection as well as the interaction between HSV-2 and HIV infection in the general population.

Nigeria has one of the highest HIV burden in the world, it is possible that developing a nationwide campaign against HSV-2 as is currently done for HIV will help stem the HIV epidemic. There is also need to identify and adequately care for pregnant women with primary or recurrent HSV-2 infection to help reduce the deadly neonatal herpes.[13] prevention of HSV-2 and identify population at highest risk for HSV-2. The low level of awareness regarding HSV-2 and the high prevalence warrant the need to develop targeted policies related to HSV-2 screening in our antenatal services. There is need for more research work regarding HSV-2 as this will further create awareness on the burden of the diseases and its impact on HIV transmission. Nigeria has one of the highest HIV burden in the world, it is possible that developing a nationwide campaign against HSV-2 as is currently done for HIV will help stem the HIV epidemic.[47] There is also need to identify and adequately care for pregnant women with primary or recurrent HSV-2 infection to help reduce the deadly neonatal herpes.[13] prevention of HSV-2 and identify population at highest risk for HSV-2. The low level of awareness regarding HSV-2 and the high prevalence warrant the need to develop targeted policies related to HSV-2 screening in our antenatal services. The study highlighted the high prevalence of HSV-2 infection in the sampled population; HIV infected (83.3%), HIV uninfected (80.7%), rural population (74.4%) and urban population (87.4%). Factors such as knowledge about HSV-2, respondent location and spousal age, significantly affect HSV-2 serostatus with spousal age being an independent predictor of HSV type 2 infection. This study informs the need to develop strategies that is aimed at reducing HSV-2 infection. A nationwide awareness campaign in the form of health education and screening programmes for HSV-2 during antenatal period will help achieve this.[42]

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