Co-infection of Herpes Simplex Virus Type 2 and HIV Infections among Pregnant Women in Ibadan, Nigeria

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Abstract

Introduction: Genital infection with herpes simplex virus type 2 (HSV-2) facilitates the acquisition of HIV, both mutually reinforcing infection. Lifelong latent HSV-2 infection raises concerns among women of reproductive age, considering the risk of neonatal transmission. In Nigeria, screening for HSV-2 and co-infection with HIV in antenatal clinics is not routine. This study was undertaken to determine the seroprevalence and co-infection of HSV-2 and HIV among pregnant women. Methods: This was a cross-sectional study conducted at the antenatal clinic of the University College Hospital, Ibadan, between March and August 2013. A total of 270 consenting pregnant women were enrolled. The study involved collecting socio-demographic data and laboratory determination of HSV-2 immunoglobulin G (IgG) and HIV seroprevalence using type-specific third-generation enzyme-linked immunosorbent assay (DIAPRO Diagnostic Bioprobes, Milan, Italy) and Uni-Gold Recombigen/ALERE determine, respectively. Data analyses were done using SPSS version 20 (SPSS Inc., IL, USA). Results: The seroprevalence for HSV-2 type-specific IgG was 33.3% (90/270), and HIV antibodies were identified in 19.63% (53/270) of the women. The HIV co-infection was 38.8% (35/90) among HSV-2-positive women and 10% (18/180) among HSV-2-negative women. Majority of the HSV-2 positive women (62.2%, 56/90) presented in their 2nd trimester while 18.9% (17/90) in their 3rd trimester. Conclusion: The seroprevalence of HSV-2 in this pregnant population is lower than what is observed in some other Sub-Saharan African countries; however, HSV-2/HIV co-infection is high. The HSV-2-seronegative women are still susceptible to primary HSV-2 infection in pregnancy with increased risk for HIV co-infection and neonatal transmission.

Keywords: HIV, herpes simplex virus type 2, neonatal transmission, pregnancy, prevalence

Introduction

Herpes simplex virus type 2 (HSV-2) and HIV are two lifelong viral sexually transmitted infections (STIs) of global health importance. HSV-2 is the predominant cause of genital herpes,[1] although it leads to periodic recurrences of painful genital ulcers in symptomatic individuals; it is asymptomatic in about 80%–90% of individuals in whom viral reactivation and shedding occur undetected.[2] Hence, HSV-2 transmissions can remain sustainable in the general public and the prevalence rates can reach very high levels if interventions to curb the infection are not in place.

There is a direct relationship between HSV-2 and HIV prevalence, and both viruses have reciprocal biological interactions.[3,4] HSV-2 has been found to enhance HIV acquisition through dense concentration of inflammatory infiltrates of CD4-positive T lymphocyte cells in the genital tract during HSV-2 shedding.[5] Furthermore, in HIV-positive patients, infection with HSV-2 accelerates replication and genital shedding of the virus; thus, such individuals are more likely to transmit HIV.[6] Among patients with HIV infection, there is a co-infection with HSV-2 in 30%–70% of those in Europe and 50%–90% of those in Africa.[7]

In 2012, a global estimate of HSV-2-infected patients aged 15–49 years was about 417 million, of which 267 million were women and Africa having the highest burden.[8] In 2016, the...
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WHO estimated about 36.7 million people to be living with HIV/AIDS globally, with Sub-Saharan Africa accounting for two-thirds. Rates of HSV-2 infection are higher among women compared with men and among pregnant women versus non-pregnant women. Among countries in the Sub-Saharan region, the prevalence of HSV-2 infection among women of the reproductive age group ranges from 30% to 80%.

HSV-2 infection in pregnancy often manifests clinically in a manner similar to that in a non-pregnant female and most times are asymptomatic except in few cases of disseminated disease. Genital herpes among pregnant women is associated with definite risk for neonatal meningoencephalitis or disseminated herpes and mortality from neonatal herpes is well above 50% even in developed countries. Regardless of the symptoms, early detection and treatment of HSV-2 during pregnancy are invaluable in limiting the risk of vertical transmission of HSV-2 to the fetus/newborn. For women with primary HSV-2 presenting with genital ulceration at the time of labor, the risk of transmission can be reduced via cesarean section.

Co-infection with HSV-2 and HIV among pregnant women may increase the risk of maternal-to-fetal transmission of HIV by as much as 25%. While some studies report poor outcome in reduction of HIV acquisition and transmission via the use of antivirals for treatment of HSV-2, it has been suggested that high-dose antivirals may reduce HIV viral load in HSV-2/HIV coinfected patients. However, it is clear that strategies to curb the HIV pandemic via the intervention measures for HSV infection control continue to be evaluated.

The objectives of this study were to determine the seroprevalence of HSV-2 and HIV infections as well as HSV-2/HIV co-infection rate among the pregnant women.

**Methods**

**Study design**

A cross-sectional hospital-based study by design was conducted in the antenatal clinic at the University College Hospital over a 9-month period from March to August 2013. Blood samples of consenting pregnant women were collected and tested for HSV-2 immunoglobulin G (IgG) and HIV-1 antibodies. Pretested structured questionnaires were filled by trained personnel during interview with consenting women.

**Study area and population**

The University College Hospital is located in Ibadan, Southwestern Nigeria, about 145 km from Lagos and 437 km from Abuja, the Federal Capital Territory. The participants were pregnant women attending antenatal clinic in the University College Hospital. The sample size was calculated based on HSV-2 seroprevalence of 22% found among pregnant women in Senegal, in Sub-Saharan Africa, to give a 95% confidence level and margin of error of ±5%. We assessed 308 pregnant women for eligibility and excluded those already diagnosed with HSV-2 infection and those unwilling to have the blood test done. Of the 280 found eligible, 270 consenting pregnant women with ages ranging between 22 and 44 years were recruited. The main reason for nonparticipation was lack of time (n = 10).

**Informed consent**

Written informed consent was obtained following explanation of the concept of the study to each pregnant woman before their inclusion in the study. Ethical clearance was sought and obtained from the Joint Ethical committee of the University of Ibadan and University College Hospital, Ibadan, before beginning the study.

**Specimen collection and handling**

Under aseptic procedure, 5 ml of venous blood was collected by venipuncture into plain bottles and allowed to clot. The sera were separated by centrifugation at room temperature and at 3000 rpm and stored in aliquots in the freezer at −20°C.

**Laboratory investigations**

All samples were screened, using qualitative sandwich third-generation enzyme-linked immunosorbent assay (ELISA), that is, type-specific IgG against the glycoprotein-G of HSV-2 (DIAPRO Diagnostic Bioprobes, Milan, Italy). The sensitivity and specificity of this assay are about 98% and there is no risk of cross-reaction with HSV-1 and 2. The same blood specimens were screened for HIV antibody using both Uni-Gold Recombigen and ALERE determine.

**HSV-2 detection by enzyme-linked immunoassay**

A glycoprotein G-based enzyme-linked immunosorbent assay technique was used. Diluted serum samples of patients were added to wells precoated with type-specific HSV-2 glycoprotein G antigens. HSV antibodies, present in the sera, were expected to bind to the HSV antigens on the surface of the well and form immune complexes which were detected by enzyme-conjugated antihuman globulin. A chromogenic substrate was added to produce colored reaction with absorbance proportional to the concentration of the HSV antibodies. Protocol for the measurement was done according to the manufacturer’s instruction and reading was done at optical density of 450 nm with an EIA plate reader. The results ran were validated and the results were interpreted according to the manufacturer’s instruction.

**Qualitative assay for antibodies to HIV-1 and 2**

Immunochromatographic assays for qualitative detection of antibodies to HIV-1 and HIV-2 were used. The women were counseled before and after the HIV test. A fingerpick specimen (whole blood) was collected into capillary tubes aseptically. About two drops (50 μl) of sample was added onto the sample pad, and after 1 min, two drops of buffer reagent
was added to the sample pad. The result was read 20 min later. The results were interpreted according to the manufacturer’s instruction.

**Data analysis**

Standard descriptive and inferential statistical analysis was carried out using SPSS version 20 (SPSS Inc., Illinois, USA). The mean, standard deviation, and test of comparison using Student’s t-test were derived for continuous variables, while categorical variables were summarized as proportions, and further analyzed using Chi-square and Fisher’s exact test to assess the association between the variables.

**RESULTS**

**Sociodemographic characteristics of the participants**

The mean age of the 270 participants was approximately 32.3 (standard deviation [SD]: 4.8 years), with ages ranging between 22 and 44 years and more than half (55.9%) aged between 29 and 35 years. Approximately 94% of participants were married (255/270) and in a monogamous relationship (254/270). All participants had some level of education; majority (198/270 [73.3%]) had tertiary-level education while 4.4% (12/270) had only primary-level education. Approximately 66% (178/270), 26% (69/270), and 8.6% (23/270) of the participants were multiparous, primiparous, and grand multiparous (parity >5 times), respectively. More than half of these women (164 [60.7%]) were in their 2nd trimester and 21% (57) in their 3rd trimester at the time of first antenatal clinic visit. Other sociodemographic characteristics are shown in Table 1.

**Prevalence of herpes simplex virus type 2, HIV infections, and herpes simplex virus type-2/HIV co-infection rates**

Among the 270 women tested, 33.3% (90/270) were HSV-2 positive while HIV antibodies were found in 19.63% (53/270). There was a significant difference in HIV positivity between HSV-2-positive and HSV-2-negative women: approximately 39% (35/90) of HSV-2-positive pregnant women were also HIV positive, while 10% (18/180) of HSV-2-negative pregnant women were HIV positive. HSV-2-positive pregnant women were older than HSV-2-negative women mean age of 32.8 (SD: 5.1) years versus 32 (SD: 4.6) years; however, Student’s t-test showed that this was not statistically significant (P = 0.174). Majority of the HSV-2-positive women (56/90 [62.2%]) presented in their 2nd trimester and 18.9% (17/90) in their 3rd trimester.

**Socio-demographic factors associated with herpes simplex virus type-2 infection among pregnant women**

Among the women found positive for anti-HSV-2 antibody, 88.9% (80/90) were in a monogamous marriage while 11.1% (10/90) were in a polygamous marriage. There was a significant positive relationship between HSV-2 infection and polygamy (χ² = 6.511, P = 0.011). Tertiary level of education predominated among those found seropositive for HSV-2 infection (57/90 [63.3%]), while those with primary level education accounted for 7.7% (7/90). A significant association between participants’ level of education and HSV-2 infection was deduced (χ² = 7.916, P = 0.019). Fewer percentages of HSV-2-positive participants (20/90 [22.2%]) were primiparous, 63.3% (57/90) were multiparous, and 14.4% (13/90) were grand multiparous (parity >5). Parity was found to be significantly associated with the patients’ HSV-2 infection status (χ² = 6.290, P = 0.043). Other sociodemographic factors associated with HSV-2 infection are shown in Table 2.

**DISCUSSION**

Substantially higher rates of HSV-2 have been observed in Sub-Saharan Africa, where prevalence in adults ranges from 30% to 80% in women and from 10% to 50% in men. The seroprevalence of HSV-2 infection among pregnant women in this study was 33.3% and is in keeping with epidemiological studies in developing countries. When compared with similar studies in other parts of Nigeria, it is lower than the 77.8% reported in Enugu by Ojinmah et al. and 44.3% observed in Benin by Kalu.

Higher prevalence rates have been reported within Africa and Europe. In some Sub-Saharan African countries, lower rates were reported, particularly in regions with higher rates.
### Table 2: Sociodemographic factors associated with herpes simplex virus type 2 infection among pregnant women

| Variable                  | Presence of HSV-2 IgG | \( \chi^2 \) | \( P \) |
|---------------------------|-----------------------|---------------|----------|
|                           | Positive, \( n (%) \) | Negative, \( n (%) \) |               |
| Age group (years)         |                       |               |          |
| 22-28                     | 17 (18.9)             | 39 (21.7)     | 2.095    | 0.351 |
| 29-35                     | 45 (50.0)             | 106 (58.9)    | 31.740   |       |
| \( \geq 35 \)             | 28 (31.1)             | 35 (19.4)     |          |       |
| Type of family            |                       |               |          |
| Monogamous                | 80 (88.9)             | 174 (96.7)    | 6.511    | 0.011*|
| Polygamous                | 10 (11.1)             | 6 (3.3)       |          |       |
| Level of education        |                       |               |          |
| Primary                   | 7 (7.7)               | 5 (2.7)       | 7.916    | 0.019*|
| Secondary                 | 26 (28.9)             | 34 (18.9)     |          |       |
| Tertiary                  | 57 (63.3)             | 141 (78.3)    |          |       |
| Marital status            |                       |               |          |
| Married                   | 84 (93.3)             | 171 (95)      | 0.318    | 0.380 |
| Single                    | 6 (6.7)               | 9 (5)         |          |       |
| Employment status         |                       |               |          |
| Employed (government/private) | 27 (30.0)       | 77 (42.8)     | 4.185    | 0.123 |
| Self employed             | 42 (46.7)             | 67 (37.2)     |          |       |
| Unemployed                | 21 (23.3)             | 36 (20.0)     |          |       |
| Religion                  |                       |               |          |
| Christian                 | 72 (80)               | 137 (76.1)    | 0.519    | 0.288 |
| Islam                     | 18 (20)               | 43 (23.9)     |          |       |
| Gestational age           |                       |               |          |
| 1st trimester             | 17 (18.9)             | 32 (17.8)     | 0.405    | 0.817 |
| 2nd trimester             | 56 (62.2)             | 108 (60.0)    |          |       |
| 3rd trimester             | 17 (18.9)             | 40 (22.2)     |          |       |
| Parity                    |                       |               |          |
| Primiparous               | 20 (22.2)             | 49 (27.2)     | 6.290    | 0.043*|
| Multiparous               | 57 (63.3)             | 121 (67.2)    |          |       |
| HIV status                |                       |               |          |
| Positive                  | 35 (38.9)             | 18 (10)       | 31.740   | <0.001*|
| Negative                  | 55 (61.1)             | 162 (90)      |          |       |

*Significant at 5% level of significance. HSV-2: Herpes simplex virus type 2, IgG: Immunoglobulin G

lower HIV prevalence rates\[^{23,25}\] when compared to the index study (19.6%). This further emphasizes the synergistic relationship between both HIV and HSV infections. Likewise, lower rates have been reported in developed countries such as North America,\[^{11}\] Western European countries,\[^{26}\] United Kingdom,\[^{27}\] Australia,\[^{28}\] and Asian countries.\[^{29,30}\]

The high variability in HSV-2 prevalence rates can be attributed to sociodemographic factors and sexual risk factors. The laboratory test methods employed may also contribute to the variation observed. Sensitivity, specificity, and positive predictive value are higher in type-specific serology and polymerase chain reaction methods as compared with others.\[^{1,8,10,31}\]

When compared with the prevalence rates among women in Sub-Saharan African countries, the HSV-2 seroprevalence rate in this pregnant population places it on the lower part of the scale; thus, a lower probability of neonatal transmission from reactivated genital herpes can be inferred. On the contrary, this also suggests that a higher percentage of our pregnant population are currently seronegative and susceptible to primary HSV-2 infection which if acquired during pregnancy may lead to increased susceptibility to HIV co-infection and high maternal-to-fetal transmission of both viruses.

Recent findings reveal that primary HSV-2 infection of the mother is the most important factor for the transmission of genital herpes from mother to fetus/newborn.\[^{11-16}\] Besides high concentrations of activated CD4-positive T cells, which are target cells for HIV in the genital area, genital ulceration and viral shedding occur most frequently in the 1\(^{st}\) year of HSV-2 infection.\[^{32,33}\] Therefore, by inference, a pregnant woman who acquires primary genital herpes in the latter half of pregnancy rather than before pregnancy is at greater risk of transmitting these viruses to her newborn. Similarly, it is pertinent to identify asynchronous recurrent HSV-2 infection in pregnancy as such women are at risk of viral shedding during delivery.\[^{11-17}\] Of the asymptomatic HSV-2-seropositive women identified in our study, majority (56/90 [62.2%]) presented in their 2\(^{nd}\) trimester while 18.9% (17/90) in their 3\(^{rd}\) trimester.

Evidence of clinical and biological studies has shown that HSV-2 is a significant driver of the HIV pandemic as the epidemiological factors determining the persistent spread of both infections are similar.\[^{1,6,12,16}\] Approximately 20% (53/270) of the study participants were seropositive for HIV antibodies. The prevalence of HIV positivity was found to be significantly higher in HSV-2 positive pregnant women than in HSV-2 negative pregnant women. Among the 90 pregnant women found to be HSV-2 positive, a significant number (35/90 [38.9%]) were coinfected with HIV (\(P^2 = 31.740, P < 0.001\)). High co-infection rate of 65.6% has been found among pregnant women in Benin,\[^{19}\] 73% among HIV-positive patients in Brazil,\[^{34}\] and 91% in Central African Republic. However, this varied with low co-infection rate of 2.8% found in a locality in Jos by Mawak \[^{et al.}\][35] and 7% in a town in Senegal.\[^{24}\] A lower prevalence of HIV in the two latter communities may be responsible for the variations observed.

Demographic factors associated with HSV-2 infection include age, level of education, type of family, and parity. Increasing age has been associated with HSV-2 infection.\[^{10,11,20,23}\] Although this study did not show a significant variation in HSV-2 infection between age groups (\(\chi^2 = 2.095, P = 0.351\)), the highest frequency of HSV-2 infection was found among the 29–35 years’ age group (50%) while women above 35 years represented 31% of the HSV-2-positive women. This may be due to the higher probability of acquiring the infection with increasing cumulative years of sexual exposure. This finding is consistent with similar studies among pregnant women,\[^{23,24,30}\] where majority of those found to be seropositive for HSV-2 were above 30 years of age. However, it varied from a report in India, by Biswas \[^{et al.}\][39] where the 22–25 years’ age group
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The HSV-2 prevalence rate in this study suggests that a greater number of our HSV-2 seronegative women are at risk of primary HSV-2 infection with increased susceptibility to HIV co-infection. Findings in literature suggest that newly acquired HSV-2 infection is associated with an increased frequency and severity of genital ulceration, inflammation, and viral shedding in the genital tract. 

Combination prevention for HIV is the current intervention strategy and the concept was borne from the realization that a combination of different intervention approaches was necessary for the sustainable control of the HIV pandemic. Therefore, control measures for HSV-2/HIV infection is a logical approach as the synergistic effect of both viruses implies that the control of HSV-2 can be an effective method of HIV prevention, thus bringing down the HIV pandemic to a low-level endemicity.

Furthermore, the sustainable development goals place emphasis on maternal and child health with recognition for the importance of women- and girl-centered approaches, especially as its intersection with the issues of STIs is on the increase. Therefore, combination prevention programs aimed at pregnant women/women of reproductive age will be a good strategy. In developing countries, a good way to start includes adequate funding to generate meaningful data for advocacy and interventions, improved access to treatment services for HSV-2 infection, HIV and other STIs, structural and behavioral changes, and capacity development.

Our study adds to the scarce information available concerning HSV-2/HIV co-infection rates among the pregnant population. However, it is limited to a sample of pregnant women in a tertiary health institution in southwestern Nigeria which is not representative of all pregnant women in Nigeria.

**CONCLUSION**

The HSV-2 prevalence rate in this study suggests that a greater number of our HSV-2-seronegative women are at risk of primary HSV-2 infection in pregnancy and increased susceptibility to HIV co-infection. Therefore, these seronegative women are at greater risk for maternal-fetal transmission of HSV-2, if newly acquired genital herpes infection occurs near the time of delivery.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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