

# Co-existence of Herpes simplex virus type 2 and two other oncoviruses is associated with cervical lesions in women living with HIV in South-Western Nigeria

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## Abstract

**Background:** The prevalence of Herpes simplex virus type 2 (HSV-2) in cervical lesions is under-reported, especially in Human immunodeficiency virus (HIV), Epstein-Barr virus (EBV) and Human Papillomavirus (HPV) infected persons.

**Objectives:** This study determined the prevalence of viral mono-infections, co-infections and squamous cell intraepithelial lesions (SIL) in HIV seropositive (HIV+) and HIV seronegative (HIV-) women.

**Methods:** This study included HIV+ and HIV- women (105 each). Cervical smears and viral antibodies were evaluated by Papanicolaou's technique and ELISA method, respectively.

**Results:** The prevalence of HSV-2, HPV and EBV infections, and SIL were higher in HIV+ women (75.2, 41.9, 41 and 32.4%) than in HIV- women (45.7, 26.7, 26.7 and 13.3%) at  $p < 0.0001$ ,  $p = 0.029$ ,  $0.041$  and  $0.002$ , respectively. Higher prevalence of viral mono-infection and tri-infection was observed in HIV+ women (43.8 and 24.8%) than in HIV- women (27.6 and 8.6%) at  $p = 0.021$ , and  $0.003$ , respectively. The prevalence of SIL was also higher in HIV+ women with viral mono-infection, bi-infection and tri-infection (15.2, 42.9, and 53.8%) than in HIV- women (6.9, 12.5, and 44.4%) at  $p = 0.468$ ,  $0.041$ , and  $0.711$ , respectively.

**Conclusion:** This study suggests that the high prevalence of SIL in HIV+ women could be associated with viral co-infections.

**Keywords:** Epstein-Barr virus; human immunodeficiency virus; human papilloma virus; herpes simplex virus type 2; cervical lesion.

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## Introduction

About half a million new cases of cervical cancer (Ca) are reported each year while approximately 49% of these cases result in death worldwide<sup>1</sup>. The reason for the higher prevalence of the disease in developing countries than developed countries is yet to be fully explained. There are varied risk factors associated with Ca but Human Papillomavirus infection remains the major risk factor. However, not all infected women develop the Ca<sup>2</sup>. In West Africa, the prevalence of HPV DNA

in atypical squamous cells of unknown significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous cell intraepithelial lesion (HSIL) and invasive Ca (ICa) is about 26, 67, 85 and 89%, respectively<sup>3</sup>. However, the risk factors associated with the remaining 74, 33, 15, and 11% of ASCUS, LSIL, HSIL and ICa, respectively are insufficiently accounted for. Immunosuppression, especially due HIV infection, is associated with high prevalence of cervical lesions<sup>4,5</sup>. Studies have shown that HPV clearance is lower in HIV+ women than in HIV- women<sup>6,7</sup>, such individuals are also at risk of acquiring other oncogenic viruses such as EBV and HSV-2<sup>8</sup>. This may explain why 25% of HIV+ women with LSIL and 12-30% of HIV+ women with HSIL still develop HSIL and ICa, respectively despite receiving antiviral therapy<sup>9-12</sup>. The reason for the progression in disease state is yet to be fully explained. Smith et al. stated that HSV-2 sero-

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positive women with normal cytology have significant higher risk for the disease than HPV DNA positive women<sup>13</sup>. Some HPV+ Ca have been found to be positive for HSV-2 antibodies<sup>14</sup>. Interestingly, Bashyai et al. observed increasing antibody titre and prevalence of HSV-2 from LSIL (11%), HSIL (33%) to Ca (40%)<sup>15</sup>. It is believed that HSV-2 associated chronic cervicitis may facilitate EBV entry<sup>16</sup>. The pooled prevalence of EBV-DNA is 3-29, 21-49, and 44-70% in LSIL, HSIL, and Ca, respectively<sup>17-21</sup>. This also implicates EBV in cervical carcinogenesis. This study determined the prevalence of viral mono-infection through tri-infection as correlates of higher SIL in HIV+ women in a developing country.

## Methods

### Sample collection, handling and assays

This comparative cross-sectional study was carried out between the months of April (2017) and June (2018) at Abeokuta metropolis, Ogun State, South-Western Nigeria. Considering that Ogun State prevalence survey for HIV is estimated at 1.4%<sup>22</sup>, a total of 105 HIV+ participants were consecutively selected from the HIV Testing and Counseling Clinic at State Hospital Ijaiye, Abeokuta. This study also included 105 HIV- women; health workers and those visiting the Family Planning Clinic in the same hospital. HIV status was confirmed by testing for HIV-1 and HIV-2 antibodies in peripheral blood (using commercial kits from Qingdao Hightop Biotech Co. Ltd, China) by the ELISA method (Cut-off value = 1.854). According to manufacturer's instruction, we also assessed clear sera for IgG and IgM antibodies against EBV (using commercial kits from Calbiotech Inc, El Cajon, USA), HPV (using commercial kits from Qingdao Hightop Biotech Co. Ltd, China) and HSV-2 (using commercial kits from Qingdao Hightop Biotech Co. Ltd, China, Calbiotech Inc, El Cajon, USA). External genitalia were dilated using speculum and the cervix was scraped using cytobrush. The cytobrush was used in making smears and the smears were stained by Papanicolaou's and Field's techniques. The stained cervical smears were classified based on the Bethesda system: 1. Negative for intraepithelial lesion or malignancy (NILM; Normal and Cervicitis), 2. ASCUS, 3. LSIL, and 4. HSIL. Participants with abnormal Pap smear result were counseled and referred to gynecologist.

Interviewer based questionnaire was used to collect socio-economic and clinical demographics: age, marital status, family type, tribe, educational level, residency,

religion, occupation, economic status (Low < 18,000 minimum wage, middle= 18,000 to 53000 and High ≥ 54,000) smoking status and alcohol consumption, age at sex debut, parity, sexual behaviour, medical history, number of sex partners, oral sex, type of contraceptives used, vaginal bleeding after sexual intercourse, genital ulcer, itching and burning sensation around the vulva, vaginal discharge, pelvic pain, duration of antiretroviral therapy and uptake of cervical screening.

### Data analysis

The sociodemographic data obtained from the questionnaire and the test results were coded as 0 (reference) and 1 (depending on the number of sub-variable) in excel, exported into SPSS (version 23) and analyzed in descending sorting order of categorical targets. Binary logistic regression analysis was used to assess the relationship between some sub-variables. Chi-square/Fisher exact test was used to compare viral infections and Pap smear result (SIL) between HIV+ and HIV- participants in relation to some demographic characteristics. Pearson's correlation was used to assess the relationship between viral infection and cervical lesions. Significant levels were set at  $p \leq 0.05$ .

### Ethical approvals

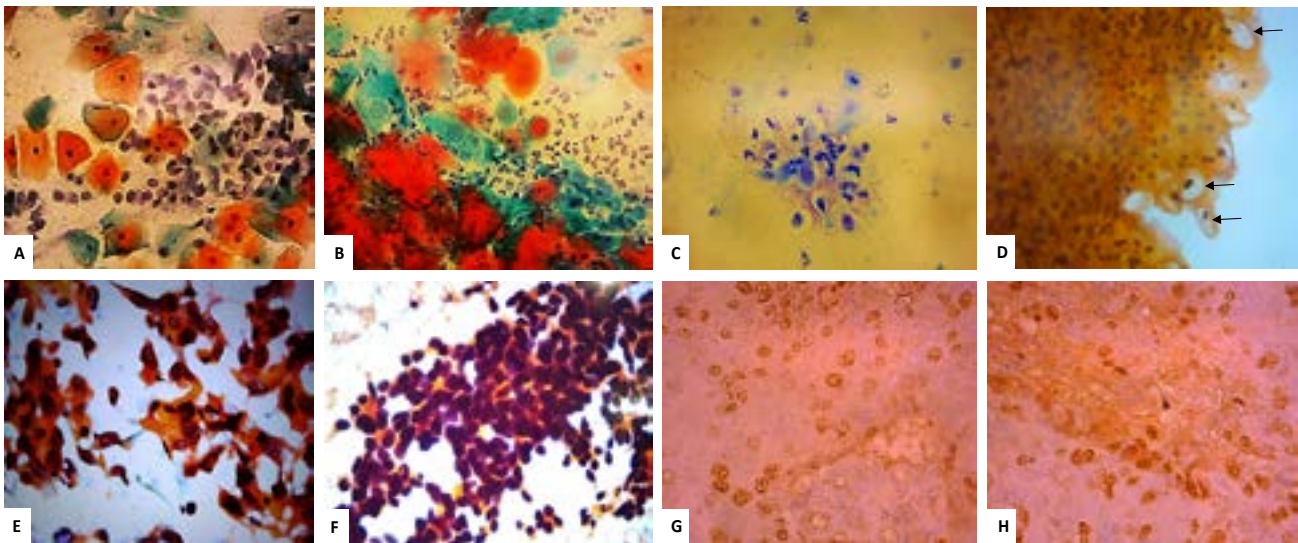
Ethical clearances were obtained from State Hospital Abeokuta Research Ethics Services (SHA/RES/VOL.2/147) and Babcock University Health Research Ethics Committee (BUHREC 353/16) and written informed consents were obtained from participants. All protocols were carried out in line with the guidelines of the ethics committees.

### Result

This study determined the prevalence of viral mono-infections and co-infections, and cervical lesions in sexually active HIV+ (mean age=  $41.55 \pm 11.71$  years) and HIV- (mean age=  $39.45 \pm 11.16$  years) participants ( $p = 0.08$ ). It included HIV+ participants who were receiving highly active anti-retroviral therapy at HIV Testing and Counseling Clinic, State Hospital Ijaiye, Abeokuta. The HIV- participants were apparently healthy women with no history of cervical lesions. The prevalence of pre-cancerous lesions (ASCUS, LSIL and HSIL; figure 1) was significantly higher in HIV+ women (51.3%) than in HIV- women (24.8%) ( $p = 0.0001$ ). Overall, the prevalence of viral co-infections was higher in HIV+ women (88.6%) than in HIV- women (33.4%) at  $p < 0.0001$ . The prevalence of HSV-2, HPV and EBV infections, and SIL were higher in HIV+ women (75.2,

41.9, 41 and 32.4%) than in HIV- women (45.7, 26.7, 26.7 and 13.3%) at  $p < 0.0001$ ,  $p = 0.029$ ,  $p = 0.041$  and  $p = 0.002$ ). Result showed that there was a significant correlation between viral infection and cervical lesions both in HIV+ women ( $r = 0.363$ ,  $p = 0.000$ ) and HIV- women ( $p = 0.000$ ). However, significant positive relationship between extent of viral infection (mono-, bi- and tri-infection) and cervical lesions was only seen in HIV+ women ( $p = 0.040$ ) while insignificant positive relationship was seen in HIV- women ( $p = 0.326$ ). Statistics revealed that the prevalence of viral mono-infection was higher in HIV+ women (43.8%) than in HIV- women (27.6%;  $p = 0.021$ ). However, the prevalence of

SIL in viral mono-infection was insignificantly higher in HIV+ women (15.2%) than in HIV- women (6.9%;  $p = 0.468$ ). No significant difference was observed in the prevalence of viral bi-infection between HIV+ (21%) and HIV- women (22.9%;  $p = 0.737$ ). Interestingly the prevalence of SIL in viral bi-infection was significantly higher in HIV+ women (42.9%) than in HIV- women (12.5%;  $p = 0.041$ ). Although, the prevalence of viral tri-infection was higher in HIV+ women (24.8%) than in HIV- women (8.6%;  $p = 0.003$ ), no significant difference was observed when the prevalence of SIL was compared between HIV+ (53.8%) and HIV- women (44.4%) with viral tri-infections ( $p = 0.711$ ).



**Figure 1: Photomicrographs of stained normal and abnormal cervical smears and abnormal tissue sections**

Figure 1: Smear A (NILM; X100), B (ASCUS with inflammatory background; X100), D (LSIL with HPV koilocytes, marked by arrows; X400), E and F (HSIL; X400) were stained by Papanicolaou's technique while C (LSIL; X400) was stained by Field's staining technique. Section G (HSIL associated with HIV/EBV/HPV/HSV-2; X100) stain moderately positive for ki67 compared with the high staining of section H (HSIL associated with EBV/HPV/HSV-2; X100).

### **Influence of demographics on prevalence of SIL and viral infections**

Descriptive statistics showed that the prevalence of SIL relatively increased with age both in HIV+ and HIV- participants with the peak prevalence in the age group of 50-59 years. The prevalence of EBV infection increased with age in HIV+ participants while the prevalence of EBV infection peaked in the age group of 30-39 years in HIV- participants and decreased afterwards. A significant difference in EBV infection was observed between HIV+ and HIV- participants in the age range of 50-59 years. The prevalence HPV infection relatively decreased with age in HIV+ and HIV- participants. A significant difference in HPV infection was also observed between HIV+ and HIV- participants in the age group of 30-39 years. A significant correlation was also observed between age and acute HSV-2 infection

( $r = 0.12$ ,  $p = 0.03$ ) in both groups. The prevalence of HSV-2 infection relatively decreased with age in HIV+ participants while the prevalence of the virus changes across age groups in HIV- women but peaked in the age group of 40-49 years. Significant differences in HSV-2 infection were also observed between HIV+ and HIV- participants in the age group of 20-39 years. Among the married women and women in polygamous marriages, the prevalence of SIL, HPV and HSV-2 infections were higher in HIV+ participants than their HIV- counterparts. Bivariate analysis revealed that the risk of EBV and HPV infection among HIV+ women in polygamous marriages were 3.26 and 4.67 (95% CI: 0.28-38.48 and 0.39-55.48) at  $p = 0.35$  and  $0.22$ , respectively when compared with women who have never been married. The odd ratio for both infections were lower among HIV+ women in monogamous marriage

0.79 and 2.00 (95% CI: 0.35-1.79 and 0.89-4.51) at  $p=0.58$  and  $0.95$ , respectively when compared with women who have never been married. Among women of Yoruba tribe, HIV+ participants had a significantly higher prevalence of HPV and HSV-2 infections than their HIV- counterparts. The number of participants with post basic (secondary school) education was lower among HIV+ participants than their HIV- counterparts ( $p=0.003$ ). The HIV+ participants with only basic education and those living in urban areas had a significantly higher prevalence of SIL, HPV and HSV-2 infections than their HIV- counterparts. The HIV+ women who were Christians had a significantly higher prevalence of SIL, EBV and HSV-2 infections than HIV-women. Significantly higher prevalence of HPV infection was observed in HIV+ Muslims than HIV- Muslims. The prevalence of participants with low income was higher in HIV+ participants compared with HIV- participants ( $p < 0.0001$ ). The HIV+ participants who with low income had significantly higher prevalence of SIL, EBV and HSV-2 infections than HIV- participants. The prevalence of SIL and HSV-2 infection were higher in HIV+ multiparous women than their HIV- counterparts. In HIV+ women, although the prevalence of EBV and HPV were higher among those who had first sexual intercourse at  $\leq 18$  years, these participants surprisingly had lower prevalence of SIL than those who had their sex debut at  $\geq 18$  years. The HIV+ women with sex debut at  $\leq 21$  years had higher prevalence of HSV-2 than their HIV- counterparts. The HIV+ debutants at  $\leq 18$  years and 19-21 years had higher prevalence of EBV and HPV infections, respectively when compared with HIV- debutants. The odd ratio of HPV infection in HIV+ debutants at 19-21 years is 44.97 (95% CI: 1.80-

1121) at  $p=0.02$ . Participants living with HIV who use only hormonal contraceptives had a significantly higher HPV infection when compared with their HIV- counterparts while HIV+ participants who intermittently use condom had significantly higher prevalence of EBV and HSV-2 infections than their HIV- participants. The HIV+ participants who had multiple sexual partners had significantly higher prevalence of SIL and HSV-2 infection than their HIV- participants. Significantly higher prevalence of HSV-2 infection was observed in HIV+ participants with vulval itching, vaginal discharge and pelvic pain than HIV- participants. The prevalence of EBV and HPV infections in HIV+ women who had HSIL were significantly higher than that of HIV-women. The prevalence of HPV and HSV-2 is higher in HIV+ women who had cervicitis and ASCUS, respectively than in HIV- women (table 1). Among HIV+ participants, significant associations were observed between EBV infection and genital ulcers in HIV+ women ( $p=0.02$ ). Among HIV+ women, multivariate analysis (MANOVA) revealed significant associations between EBV infection and cervical lesions ( $p=0.001$ ), HSV-2 and cervical lesions ( $p=0.03$ ), HPV infection and cervical lesions ( $p=0.001$ ), HSV-2 and EBV infection ( $p=0.001$ ), HPV and EBV infection ( $p=0.001$ ), and EBV/HPV/HSV-2 and cervical lesions ( $P=0.001$ ). Among HIV- participants, MANOVA revealed a significant association between EBV and HSV-2 infections ( $p=0.001$ ). It also revealed significant associations between EBV and Oral sex ( $p=0.05$ ), EBV infection and pelvic pain ( $p=0.02$ ), EBV/HPV/HSV-2 tri-infection and history of multiple sex partner ( $p=0.02$ ), EBV/HPV/HSV-2 tri-infection and vulval itching ( $p=0.02$ ), and cervical lesions and vaginal bleeding ( $p=0.03$ ).

**Table 1a: Socioeconomic and clinical characteristics of HIV seropositive and seronegative participants**

| Variables        | Sub-variables  | HIV+ Ppts | HIV- Ppts | Ppts with SIL |            | p-value      | EBV Infection |            | p-value      | HPV Infection |            | p-value      | HSV-2 Infection |            | p-value      |
|------------------|----------------|-----------|-----------|---------------|------------|--------------|---------------|------------|--------------|---------------|------------|--------------|-----------------|------------|--------------|
|                  |                | N= 105    | N= 105    | HIV+ n= 34    | HIV- n= 14 |              | HIV+ n= 43    | HIV- n= 28 |              | HIV+ n= 44    | HIV- n= 28 |              | HIV+ n= 79      | HIV- n= 48 |              |
| Age              | 20-29          | 39 (37.1) | 38 (36.2) | 9 (23.1)      | 5 (13.2)   | 0.377        | 15 (38.5)     | 10 (26.3)  | 0.332        | 17 (43.6)     | 13 (34.2)  | 0.485        | 32 (82.1)       | 20 (52.6)  | <b>0.008</b> |
|                  | 30-39          | 31 (29.5) | 42 (40.0) | 9 (29.0)      | 5 (11.9)   | 0.076        | 12 (38.7)     | 14 (33.3)  | 0.805        | 14 (45.2)     | 8 (19.0)   | <b>0.021</b> | 23 (74.2)       | 17 (40.5)  | <b>0.009</b> |
|                  | 40-49          | 25 (23.8) | 13 (12.4) | 10 (40.0)     | 2 (15.4)   | 0.158        | 11 (44.0)     | 3 (23.1)   | 0.294        | 10 (40.0)     | 6 (24.0)   | 0.742        | 17 (68.0)       | 8 (61.5)   | 0.730        |
|                  | 50-59          | 9 (8.6)   | 11 (10.5) | 6 (66.7)      | 2 (18.2)   | 0.065        | 5 (55.6)      | 1 (9.1)    | <b>0.050</b> | 3 (33.3)      | 1 (11.1)   | 0.285        | 6 (66.7)        | 3 (27.3)   | 0.175        |
|                  | 60-69          | 1 (1.0)   | 1 (1.0)   | 0 (0.0)       | 0 (0.0)    | 1.000        | 0 (0.0)       | 0 (0.0)    | 1.000        | 0 (0.0)       | 0 (0.0)    | 1.000        | 1 (100)         | 0 (0.0)    | 1.000        |
| Marital Status   | Never Married  | 3 (2.9)   | 44 (41.9) | 0 (0.0)       | 7 (70.5)   | 1.000        | 2 (66.7)      | 11 (25.0)  | 0.181        | 2 (66.7)      | 18 (40.9)  | 0.567        | 3 (100)         | 22 (50.0)  | 0.237        |
|                  | Married        | 72 (68.6) | 46 (43.8) | 24 (86.1)     | 6 (52.2)   | <b>0.017</b> | 28 (38.9)     | 13 (28.3)  | 0.836        | 30 (41.7)     | 6 (13.0)   | <b>0.001</b> | 50 (69.4)       | 19 (41.3)  | <b>0.004</b> |
|                  | D/S/W          | 30 (28.6) | 15 (14.3) | 10 (93.3)     | 1 (46.7)   | 0.070        | 13 (43.3)     | 4 (26.7)   | 0.341        | 12 (40.0)     | 4 (26.7)   | 0.514        | 26 (86.7)       | 7 (46.7)   | <b>0.010</b> |
| Family Type      | Never Married  | 3 (2.9)   | 43 (41.1) | 0 (0.0)       | 7 (16.3)   | 1.000        | 2 (66.7)      | 11 (25.6)  | 0.188        | 2 (66.7)      | 18 (41.9)  | 0.572        | 3 (100)         | 22 (51.2)  | 0.239        |
|                  | Poly           | 52 (49.5) | 28 (26.7) | 19 (36.5)     | 3 (10.7)   | <b>0.018</b> | 21 (40.4)     | 10 (35.7)  | 0.811        | 25 (48.1)     | 3 (10.7)   | <b>0.001</b> | 38 (73.1)       | 12 (42.9)  | <b>0.015</b> |
| Tribe            | Mono           | 50 (47.6) | 34 (32.4) | 15 (30.0)     | 4 (11.8)   | 0.065        | 20 (40.0)     | 7 (20.6)   | 0.095        | 17 (34.0)     | 7 (20.6)   | 0.223        | 38 (76.0)       | 14 (63.6)  | <b>0.003</b> |
|                  | Igbo           | 3 (2.9)   | 22 (21.0) | 2 (66.7)      | 3 (13.6)   | 0.091        | 2 (66.7)      | 6 (27.3)   | 0.231        | 2 (66.7)      | 7 (31.8)   | 0.530        | 2 (66.7)        | 13 (59.1)  | 1.000        |
|                  | Yoruba         | 98 (93.3) | 54 (51.4) | 31 (31.6)     | 10 (18.5)  | 0.089        | 40 (40.8)     | 18 (33.3)  | 0.388        | 41 (41.8)     | 11 (20.4)  | <b>0.008</b> | 77 (78.6)       | 25 (46.3)  | <b>0.000</b> |
|                  | Hausa          | 2 (1.9)   | 5 (4.8)   | 0 (0.0)       | 0 (0.0)    | 1.000        | 1 (50.0)      | 0 (0.0)    | 1.000        | 1 (50.0)      | 1 (20.0)   | 1.000        | 0 (0.0)         | 1 (20.0)   | 1.000        |
| Education        | Others         | 2 (1.9)   | 24 (22.9) | 1 (50.0)      | 1 (4.2)    | 0.151        | 1 (50.0)      | 6 (25.0)   | 0.474        | 0 (0.0)       | 11 (45.8)  | 0.492        | 0 (0.0)         | 9 (37.5)   | 0.529        |
|                  | N.F.E.         | 11 (10.5) | 6 (5.7)   | 3 (27.3)      | 1 (16.7)   | 1.000        | 6 (54.5)      | 1 (16.7)   | 0.304        | 5 (45.5)      | 3 (50.0)   | 1.000        | 11 (100)        | 4 (66.7)   | 0.110        |
|                  | Primary        | 54 (51.4) | 27 (25.7) | 20 (37.0)     | 4 (14.8)   | 0.043        | 19 (35.2)     | 8 (29.6)   | 0.803        | 20 (37.0)     | 10 (37.0)  | 1.000        | 38 (70.4)       | 15 (55.6)  | 0.220        |
|                  | Secondary      | 31 (29.5) | 46 (43.8) | 9 (29.0)      | 3 (6.5)    | <b>0.012</b> | 12 (38.7)     | 10 (21.7)  | 0.128        | 16 (51.6)     | 10 (21.7)  | <b>0.013</b> | 23 (74.2)       | 21 (45.7)  | <b>0.019</b> |
| Residence        | Post-Secondary | 9 (8.6)   | 26 (24.8) | 2 (22.2)      | 6 (23.1)   | 1.000        | 6 (66.7)      | 9 (34.6)   | 0.129        | 3 (33.3)      | 9 (34.6)   | 1.000        | 7 (77.8)        | 12 (46.2)  | 0.135        |
|                  | Urban          | 100(95.2) | 64 (61.0) | 32 (32.0)     | 9 (14.1)   | <b>0.010</b> | 41 (64.1)     | 19 (29.7)  | 0.184        | 40 (40.0)     | 15 (23.4)  | <b>0.041</b> | 76 (76.0)       | 32 (50.0)  | <b>0.001</b> |
| Religion         | Rural          | 5 (4.8)   | 41 (39.0) | 2 (40.0)      | 5 (100)    | 0.160        | 2 (40.0)      | 9 (22.0)   | 0.580        | 4 (80.0)      | 13 (31.7)  | 0.055        | 3 (60.0)        | 16 (39.0)  | 0.635        |
|                  | Christian      | 65 (61.9) | 93 (88.6) | 19 (29.2)     | 12 (12.9)  | <b>0.014</b> | 29 (44.6)     | 22 (23.7)  | <b>0.009</b> | 25 (38.5)     | 25 (26.9)  | 0.164        | 48 (27.7)       | 40 (43.0)  | <b>0.000</b> |
| Income Status    | Muslim         | 40 (38.1) | 12 (11.4) | 15 (37.5)     | 2 (16.7)   | 0.484        | 14 (35.0)     | 6 (50.0)   | 0.500        | 19 (47.5)     | 3 (25.0)   | <b>0.012</b> | 31 (37.5)       | 8 (66.7)   | 0.466        |
|                  | Low            | 77 (73.3) | 34 (32.4) | 25 (32.5)     | 4 (11.8)   | <b>0.033</b> | 35 (45.5)     | 7 (20.6)   | <b>0.019</b> | 34 (44.2)     | 11 (32.4)  | 0.297        | 59 (76.6)       | 16 (47.1)  | <b>0.012</b> |
|                  | Middle         | 22 (21.0) | 51 (48.6) | 6 (27.3)      | 5 (9.8)    | 0.077        | 7 (31.8)      | 15 (29.4)  | 1.000        | 7 (31.8)      | 15 (29.4)  | 1.000        | 16 (72.7)       | 22 (43.1)  | <b>0.024</b> |
| Parity           | High           | 6 (5.7)   | 20 (19.0) | 3 (50.0)      | 5 (25.0)   | 0.330        | 1 (16.7)      | 6 (30.0)   | 0.647        | 3 (50.0)      | 2 (10.0)   | 0.062        | 4 (66.7)        | 10 (50.0)  | 0.652        |
|                  | Null           | 46 (43.8) | 39 (37.1) | 16 (34.8)     | 6 (15.4)   | <b>0.050</b> | 20 (43.5)     | 7 (17.9)   | <b>0.019</b> | 18 (39.1)     | 12 (30.8)  | 0.498        | 33 (71.7)       | 17 (43.6)  | <b>0.015</b> |
|                  | 1-2 children   | 6 (5.7)   | 8 (7.6)   | 2 (33.3)      | 2 (25.0)   | 1.000        | 1 (16.7)      | 4 (50.0)   | 0.301        | 4 (66.7)      | 1 (12.5)   | 0.091        | 3 (50.0)        | 6 (75.0)   | 0.580        |
| Age at sex debut | Multiparous    | 53 (50.5) | 58 (55.2) | 16 (30.2)     | 6 (10.3)   | <b>0.016</b> | 22 (41.5)     | 17 (29.3)  | 0.233        | 22 (41.5)     | 15 (25.9)  | 0.107        | 43 (81.1)       | 25 (43.1)  | <b>0.000</b> |
|                  | ≤18years       | 26 (24.8) | 40 (38.1) | 6 (23.1)      | 4 (10.0)   | 0.175        | 14 (53.8)     | 8 (20.0)   | <b>0.007</b> | 13 (50.0)     | 11 (27.5)  | 0.074        | 19 (73.1)       | 17 (42.5)  | <b>0.023</b> |
|                  | 19-21 years    | 48 (45.7) | 43 (41.0) | 14 (29.2)     | 6 (14.0)   | 0.127        | 17 (35.4)     | 11 (25.6)  | 0.367        | 17 (35.4)     | 5 (11.6)   | <b>0.013</b> | 36 (75.0)       | 19 (44.2)  | <b>0.005</b> |
|                  | ≥22 years      | 31 (29.5) | 22 (21.0) | 14 (45.2)     | 4 (18.2)   | 0.076        | 12 (38.7)     | 9 (40.9)   | 1.000        | 14 (45.2)     | 7 (31.8)   | 0.393        | 24 (77.4)       | 12 (54.5)  | 0.134        |
| Contraceptive    | Hormonal       | 51 (48.6) | 40 (38.1) | 15 (29.4)     | 6 (15.0)   | 0.135        | 21 (41.2)     | 13 (32.5)  | 0.513        | 21 (41.2)     | 5 (12.5)   | <b>0.005</b> | 41 (80.0)       | 18 (45.0)  | 1.000        |
|                  | Condom         | 54 (51.4) | 65 (61.9) | 18 (33.3)     | 8 (12.3)   | 0.001        | 22 (40.7)     | 15 (23.1)  | <b>0.048</b> | 23 (42.6)     | 23 (35.4)  | 0.454        | 38 (70.4)       | 30 (46.2)  | <b>0.010</b> |
| Oral Sex         | No             | 104(99.0) | 89 (84.8) | 34 (32.7)     | 11(12.4)   | 0.001        | 42 (40.4)     | 25 (28.1)  | 0.095        | 44 (42.3)     | 22 (24.7)  | <b>0.015</b> | 78 (75.0)       | 39 (43.8)  | <b>0.000</b> |
|                  | Yes            | 1 (1.0)   | 16 (15.2) | 0 (0.0)       | 3 (18.8)   | 1.000        | 1 (100)       | 3 (18.8)   | 0.235        | 0 (0.0)       | 6 (37.5)   | 1.000        | 1 (100)         | 9 (56.3)   | 1.000        |
| Smoker           | No             | 105(100)  | 90 (85.7) | 34 (32.7)     | 12(13.3)   | <b>0.002</b> | 43 (41.0)     | 25 (27.8)  | 0.070        | 44 (41.9)     | 21 (23.3)  | 0.006        | 79 (75.2)       | 39 (43.3)  | <b>0.000</b> |
|                  | Yes            | 0 (0.0)   | 15 (14.3) | 0 (0.0)       | 2 (13.3)   | 1.000        | 0 (0.0)       | 3 (20.0)   | 1.000        | 0 (0.0)       | 7 (46.7)   | 1.000        | 0 (0.0)         | 9 (60.0)   | 1.000        |
| Alcohol intake   | No             | 101(96.2) | 71 (67.6) | 33 (32.7)     | 11(15.5)   | <b>0.013</b> | 43 (42.6)     | 21 (29.6)  | 0.151        | 41 (40.6)     | 20 (28.2)  | 0.107        | 76 (75.2)       | 34 (47.9)  | <b>0.000</b> |
|                  | Yes            | 4 (3.8)   | 34 (32.4) | 1 (25.0)      | 3 (8.8)    | 0.372        | 2 (50.0)      | 7 (20.6)   | 0.233        | 3 (75.0)      | 8 (23.5)   | 0.065        | 3 (75.0)        | 14 (41.2)  | 0.307        |

**Table 1b: Socioeconomic and clinical characteristics of HIV seropositive and seronegative participants**

| Variables         | Sub-variables | HIV+ Ppts | HIV- Ppts | Ppts with SIL |            | p-value | EBV infection |            | p-value | HPV Infection |            | p-value | HSV-2 Infection |            | p-value |
|-------------------|---------------|-----------|-----------|---------------|------------|---------|---------------|------------|---------|---------------|------------|---------|-----------------|------------|---------|
|                   |               | N= 105    | N= 105    | HIV+ n= 34    | HIV- n= 14 |         | HIV+ n= 43    | HIV- n= 28 |         | HIV+ n= 44    | HIV- n= 28 |         | HIV+ n= 79      | HIV- n= 48 |         |
| MSP               | No            | 43 (41.0) | 33 (31.4) | 15 (34.9)     | 8 (24.2)   | 0.143   | 21 (48.8)     | 12 (36.4)  | 0.352   | 19 (44.2)     | 7 (21.2)   | 0.051   | 32 (74.4)       | 14 (42.4)  | 0.009   |
|                   | Yes           | 62 (59.0) | 72 (68.6) | 19 (30.6)     | 6 (8.3)    | 0.002   | 22 (35.5)     | 16 (22.2)  | 0.124   | 25 (40.3)     | 21 (29.2)  | 0.204   | 47 (75.8)       | 34 (47.2)  | 0.001   |
| HUTI              | No            | 74 (70.5) | 84 (80.0) | 19 (25.7)     | 10 (11.9)  | 0.038   | 26 (35.1)     | 21 (25.0)  | 0.222   | 27 (36.5)     | 22 (26.2)  | 0.173   | 54 (73.0)       | 36 (42.9)  | 0.000   |
|                   | Yes           | 31 (29.5) | 21 (20.0) | 15 (48.4)     | 4 (19.0)   | 0.042   | 17 (54.8)     | 7 (33.3)   | 0.162   | 17 (54.8)     | 6 (28.6)   | 0.089   | 25 (80.6)       | 12 (57.1)  | 0.117   |
| Ulcers            | No            | 93 (88.6) | 98 (93.3) | 30 (32.3)     | 12 (12.2)  | 0.001   | 35 (37.6)     | 25 (25.5)  | 0.087   | 39 (41.9)     | 24 (24.5)  | 0.014   | 68 (73.1)       | 44 (44.9)  | 0.000   |
|                   | Yes           | 12 (11.4) | 7 (6.7)   | 4 (33.3)      | 2 (28.6)   | 1.000   | 8 (66.7)      | 3 (42.9)   | 0.377   | 5 (41.7)      | 4 (57.1)   | 0.650   | 11 (91.7)       | 4 (57.1)   | 0.117   |
| Vulval itching    | No            | 81 (77.1) | 80 (76.2) | 26 (32.1)     | 9 (34.6)   | 0.002   | 31 (38.3)     | 25 (31.3)  | 0.409   | 31 (38.3)     | 20 (25.0)  | 0.090   | 61 (75.3)       | 40 (50.0)  | 0.001   |
|                   | Yes           | 24 (22.9) | 25 (23.8) | 8 (33.3)      | 5 (20.0)   | 0.345   | 12 (54.5)     | 3 (12.0)   | 0.005   | 13 (54.2)     | 8 (32.0)   | 0.244   | 18 (75.0)       | 8 (32.0)   | 0.004   |
| Vaginal discharge | No            | 59 (56.2) | 61 (58.1) | 19 (32.2)     | 7 (11.5)   | 0.008   | 22 (37.3)     | 15 (24.6)  | 0.167   | 25 (42.4)     | 18 (29.5)  | 0.183   | 43 (72.9)       | 34 (55.7)  | 0.059   |
|                   | Yes           | 46 (43.8) | 44 (41.9) | 15 (32.6)     | 7 (15.9)   | 0.087   | 21 (45.7)     | 13 (29.5)  | 0.133   | 19 (41.3)     | 10 (22.7)  | 0.073   | 36 (78.3)       | 14 (31.8)  | 0.000   |
| Vaginal bleeding  | No            | 100(95.2) | 99 (94.3) | 32 (32.0)     | 10 (10.1)  | 0.000   | 40 (40.0)     | 27 (27.3)  | 0.072   | 42 (42.0)     | 28 (28.3)  | 0.054   | 74 (74.0)       | 46 (46.5)  | 0.000   |
|                   | Yes           | 5 (4.8)   | 6 (5.7)   | 2 (40.0)      | 4 (66.7)   | 0.567   | 3 (60.0)      | 1 (16.7)   | 0.242   | 2 (40.0)      | 0 (0.0)    | 0.182   | 5 (100)         | 2 (33.3)   | 0.061   |
| Pelvic Pain       | No            | 97 (92.4) | 80 (76.2) | 33 (34.0)     | 11 (13.8)  | 0.003   | 40 (41.2)     | 21 (26.3)  | 0.040   | 39 (40.2)     | 20 (25.0)  | 0.038   | 72 (74.2)       | 39 (48.8)  | 0.001   |
|                   | Yes           | 8 (7.6)   | 25 (23.8) | 1 (12.5)      | 3 (12.0)   | 1.000   | 3 (37.5)      | 7 (28.0)   | 0.673   | 5 (62.5)      | 8 (32.0)   | 0.213   | 7 (87.5)        | 9 (36.0)   | 0.017   |
| UCS               | No            | 101(96.2) | 96 (91.4) | 33 (32.7)     | 11 (11.5)  | 0.001   | 39 (38.6)     | 27 (28.1)  | 0.099   | 42 (41.6)     | 26 (27.1)  | 0.037   | 75 (74.3)       | 44 (14.6)  | 0.000   |
|                   | Yes           | 4 (3.8)   | 9 (8.6)   | 1 (20.0)      | 3 (33.3)   | 1.000   | 2 (50.0)      | 1 (11.1)   | 0.203   | 2 (50.0)      | 2 (22.2)   | 0.530   | 4 (100)         | 4 (44.4)   | 0.105   |
| Pap smear result  | None          | 36 (34.3) | 60 (57.1) | --            | --         | --      | 11 (30.6)     | 12 (20.0)  | 0.324   | 8 (22.2)      | 18 (30.0)  | 0.482   | 22 (61.1)       | 23 (38.3)  | 0.036   |
|                   | Cervicitis    | 15 (14.3) | 19 (18.1) | --            | --         | --      | 4 (26.7)      | 6 (31.6)   | 1.000   | 7 (46.7)      | 2 (10.5)   | 0.022   | 12 (80.0)       | 9 (47.4)   | 0.079   |
|                   | ASCUS         | 20 (19.9) | 12 (11.4) | --            | --         | --      | 8 (40.0)      | 3 (25.0)   | 0.465   | 8 (40.0)      | 4 (33.3)   | 1.000   | 19 (95.0)       | 7 (58.3)   | 0.019   |
|                   | LSIL          | 20 (19.9) | 8 (7.6)   | --            | --         | --      | 8 (40.0)      | 5 (62.5)   | 0.410   | 11 (55.0)     | 3 (37.5)   | 0.678   | 14 (70.0)       | 5 (62.5)   | 1.000   |
|                   | HSIL          | 14 (13.3) | 6 (5.7)   | --            | --         | --      | 12 (85.7)     | 2 (33.3)   | 0.037   | 10 (71.4)     | 1 (16.7)   | 0.050   | 12 (85.7)       | 4 (66.7)   | 0.549   |
| Infection         | None          | 12 (11.4) | 43 (41.0) | 4 (3.8)       | 5 (4.7)    | 0.092   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | EBV           | 2 (1.9)   | 3 (2.9)   | 0 (0.0)       | 0 (0.0)    | 1.000   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | HPV           | 11 (10.5) | 10 (9.5)  | 4 (3.8)       | 0 (0.0)    | 0.090   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | HSV-2         | 33 (31.4) | 16 (15.2) | 3 (2.9)       | 2 (1.9)    | 1.000   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | EBV+HSV-2     | 14 (13.3) | 15 (14.3) | 6 (5.7)       | 3 (2.9)    | 0.245   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | HPV+HSV-2     | 6 (5.7)   | 8 (7.6)   | 3 (2.9)       | 0 (0.0)    | 0.054   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | EBV+HPV       | 1 (1.0)   | 1 (1.1)   | 0 (0.0)       | 0 (0.0)    | 1.000   | --            | --         | --      | --            | --         | --      | --              | --         | --      |
|                   | EBV/HPV/HSV-2 | 26 (24.8) | 9 (8.6)   | 14 (13.3)     | 4 (3.8)    | 0.711   | --            | --         | --      | --            | --         | --      | --              | --         | --      |

Keys: N.F.E; No formal education, M.S.P; Multiple sexual partners, Sec; Secondary; D/S/W; Divorced/Separated/Widow, Abn; Abnormal, Ppt; participants, HUTI; History of urogenital infection; UCS; Uptake of cervical screening.

Prevalence of SIL in acute and chronic viral infection The ratio of chronic HPV infection and acute HPV infection in HIV+ and HIV- women were 2.7:1 and 7:1, respectively. In HIV+ women with SIL, higher prevalence of chronic HSV-2 and EBV infections (70.6% and 55.9%) were observed than acute infections (50% and 17.6%, respectively) while lower prevalence of chronic

HPV infection (23.5%) was observed than acute HPV infection (41.2%). In HIV- women with SIL, higher prevalence of chronic HPV, HSV-2 and EBV infections (21.4, 64.3 and 50%) were observed than acute infections (14.3, 35.7 and 21.4%, respectively). The number of women who were positive for both IgG and IgM antibodies were higher in HIV+ participants when compare with HIV- women (table 2).

**Table 2: Viral antibody positivity among HIV- and HIV+ participants in relation to some classes of Pap smear**

| Groups                      | N= 210    | HPV          |              | HSV-2        |              | EBV          |              | HPV IgG+IgM | HSV-2 IgG+IgM | EBV IgG+IgM |
|-----------------------------|-----------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|---------------|-------------|
|                             |           | IgG          | IgM          | IgG          | IgM          | IgG          | IgM          |             |               |             |
| <b>Cut-off value</b>        |           | <b>1.071</b> | <b>0.438</b> | <b>1.520</b> | <b>0.102</b> | <b>1.570</b> | <b>1.030</b> |             |               |             |
| <b>HIV Seropositive</b>     |           |              |              |              |              |              |              |             |               |             |
| HSIL                        | 14 (13.3) | 4 (28.6)     | 6 (42.9)     | 11 (78.6)    | 5 (35.7)     | 11 (78.6)    | 3 (11.5)     | 0 (0.0)     | 4 (28.6)      | 2 (11.5)    |
| LSIL                        | 20 (19.0) | 4 (20.0)     | 8 (40.0)     | 13 (65.0)    | 12 (60.0)    | 8 (40.0)     | 3 (18.8)     | 1 (5.0)     | 12 (60.0)     | 3 (12.5)    |
| ASCUS                       | 20 (19.0) | 7 (35.0)     | 4 (20.0)     | 17 (85.0)    | 16 (80.0)    | 8 (40.0)     | 2 (10.0)     | 3 (15.0)    | 14 (70.0)     | 2 (10.0)    |
| Cervicitis                  | 15 (14.3) | 2 (13.3)     | 7 (46.7)     | 5 (33.3)     | 7 (46.7)     | 1 (6.7)      | 3 (20.0)     | 2 (13.3)    | 0 (0.0)       | 0 (0.0)     |
| NILM                        | 36 (34.3) | 1 (2.8)      | 6 (16.7)     | 9 (25.0)     | 13 (36.1)    | 8 (40.0)     | 3 (4.8)      | 0 (0.0)     | 0 (0.0)       | 0 (0.0)     |
| <b>HIV Seronegative</b>     |           |              |              |              |              |              |              |             |               |             |
| HSIL                        | 6 (5.7)   | 1 (16.7)     | 0 (0.0)      | 4 (66.7)     | 2 (33.3)     | 2 (33.3)     | 1 (16.7)     | 0 (0.0)     | 2 (33.3)      | 1 (16.7)    |
| LSIL                        | 8 (7.6)   | 2 (25.0)     | 2 (25.0)     | 5 (62.5)     | 3 (37.5)     | 5 (62.5)     | 2 (25.0)     | 1 (12.5)    | 3 (37.5)      | 2 (25.0)    |
| ASCUS                       | 12 (11.4) | 3 (25.0)     | 1 (8.3)      | 5 (41.7)     | 3 (60.0)     | 3 (33.3)     | 1 (8.3)      | 0 (0.0)     | 1 (8.3)       | 1 (8.3)     |
| Cervicitis                  | 19 (18.1) | 1 (5.26)     | 1 (5.3)      | 4 (21.1)     | 6 (31.6)     | 5 (26.3)     | 1 (5.3)      | 0 (0.0)     | 1 (5.3)       | 0 (0.0)     |
| NILM                        | 60 (57.1) | 16 (26.7)    | 2 (3.3)      | 15 (25.0)    | 12 (20.0)    | 12 (31.7)    | 2 (3.3)      | 0 (0.0)     | 4 (6.7)       | 1 (1.7)     |
| <b>HIV+ (n=105)</b>         |           | 18 (17.1)    | 31 (29.5)    | 55 (52.4)    | 53 (50.5)    | 36 (34.3)    | 14 (10.3)    | 3 (2.9)     | 30 (28.6)     | 7 (6.2)     |
| <b>HIV- (n=105)</b>         |           | 23 (15.2)    | 6 (5.7)      | 33 (31.4)    | 26 (24.8)    | 27 (25.7)    | 7 (6.7)      | 1 (1.0)     | 11 (10.5)     | 5 (4.8)     |
| <b>HIV+ vs HIV- p-value</b> |           | 0.4865       | < 0.0001     | 0.0032       | 0.0002       | 0.2282       | 0.1664       | 0.6214      | 0.0015        | 0.7677      |



## Discussion

The prevalence of cervical lesions in HIV+ and HIV- women in this study is similar to the 56% and 12.6% reported among HIV+ and HIV- women in North Central Nigeria by Lawal et al., respectively<sup>23</sup> but lower than the 61.1% and 76% reported in HIV+ women at Tanzania and Zambia, respectively<sup>11,24</sup>. In this study, the prevalence of SIL is higher than the prevalence reported in Ethiopian HIV+ women (13.6%) and HIV- women (5.2%), respectively<sup>25</sup>. Studies have shown that HIV infection increases the risk for CIN (SIL) by 2.3 to 5 times<sup>26-28</sup>. This is similar to the findings of this study which showed that the prevalence of SIL was 2.4 times higher in HIV+ women than in HIV- women. The difference in the prevalence of SIL between HIV+ and HIV- participants could be accrued to other viral infections apart from HPV infection. Such viruses may include EBV and HSV-2. The prevalence of HSV-2 infection in this study is similar to the prevalence of 85% and 53% observed in HIV+ and HIV- participants in Uganda<sup>29</sup>. Similar to the findings of Nakku-Jobba et al., age, level of education, number of sexual partners and presence of ulcers were major correlates of HSV-2 infections<sup>29</sup>. A recent study revealed that HSV-2 seropositive women have a 2- to 9-fold<sup>30</sup> increase in the prevalence of the virus with disease state suggests that HSV-2 plays a role in cervical carcinogenesis. Additionally, the co-existence of HSV-2 and HPV is believed to be associated with higher risk for Ca. This is evident in a study which shows that the relative risk (RR) for Ca among HSV-2, HPV and HSV-2/HPV positive groups are 2.79, 2.98 and 3.44 (95% CI: 1.31-5.96, 1.23-7.20 and 1.50-7.86), respectively<sup>31</sup>.

Interestingly, the prevalence of EBV infection was associated with older age in HIV+ women while the prevalence of the virus was associated with younger age in HIV- women. The divergence in age related prevalence of the virus may account for the difference in the prevalence of SIL between the two groups. This is underscored by the fact that the prevalence of SIL in HIV+ women was associated with older age as well. The relationship between older age and prevalence of EBV has early been reported<sup>20</sup>. Unlike in HIV- women, increasing prevalence of EBV infection was associated with disease state in HIV+ women, with the highest prevalence in HSIL. Similar reports have been made in earlier studies<sup>20-21</sup>. However, these studies were not explicit on the HIV status of their entire participants.

Though the prevalence of HPV infection was associated with younger age both in HIV+ and HIV- women, its prevalence was associated with higher disease state in HIV+ women. This again demonstrates the importance of EBV and HPV persistence in cervical carcinogenesis, especially in immunocompromised individuals. Studies have shown that the absence or reduced antiviral T-cell response in HIV infection favours superinfections, co-infections, persistence, replication and reactivation of latent oncoviruses<sup>32,33</sup>. This might explain why HIV+ women had higher EBV/HPV associated SIL than HIV- women. Studies have shown that the presence of EBV latent membrane protein 1 and HPV is associated with aggressive and poorly differentiated squamous cell carcinomas phenotype<sup>20,34,35</sup>. This may be associated with the higher prevalence of SIL observed in HIV+ women. The high prevalence of SIL in HIV+ women with tri-infection could be due to immune exhaustion, since these women had a lower expression of Ki67 in HSIL than their HIV- counterparts<sup>18</sup>. Although the prevalence of HSV-2 is lower in HIV- women than their HIV+ counterparts, such HIV- women are at risk of acquiring HIV infections, especially those with acute HSV-2 infections<sup>36</sup>.

## Conclusion

This study revealed that the prevalence of viral mono-infections and co-infections, and SIL were higher in HIV+ women than in HIV- women. It also revealed that higher prevalence of SIL in viral tri-infection than in bi-infection and mono-infection both in HIV+ and HIV- women. It suggests that the high prevalence of SIL in HIV+ women could be associated with viral co-infections.

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## Conflict of interest

None declared

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