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[1]. 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[2]. 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[3]. 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[4,5]. Studies have shown that HPV clearance is lower in HIV+ women than in HIV- women[6,7], such individuals are also at risk of acquiring other oncogenic viruses such as EBV and HSV-2[8]. 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[9-12]. 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[13]. Some HPV+ Ca have been found to be positive for HSV-2 antibodies[14]. Interestingly, Bashyai et al. observed increasing antibody titre and prevalence of HSV-2 from LSIL (11%), HSIL (33%) to Ca (40%)[15]. It is believed that HSV-2 associated chronic cervicitis may facilitate EBV entry[16]. The pooled prevalence of EBV-DNA is 3-29, 21-49, and 44-70% in LSIL, HSIL, and Ca, respectively[17-21]. 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%[22], 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 ± 11.71 years) and HIV- (mean age= 39.45 ± 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 prevalnce 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 compare between HIV+ (53.8%) and HIV- women (44.4%) with viral tri-infections (p= 0711).

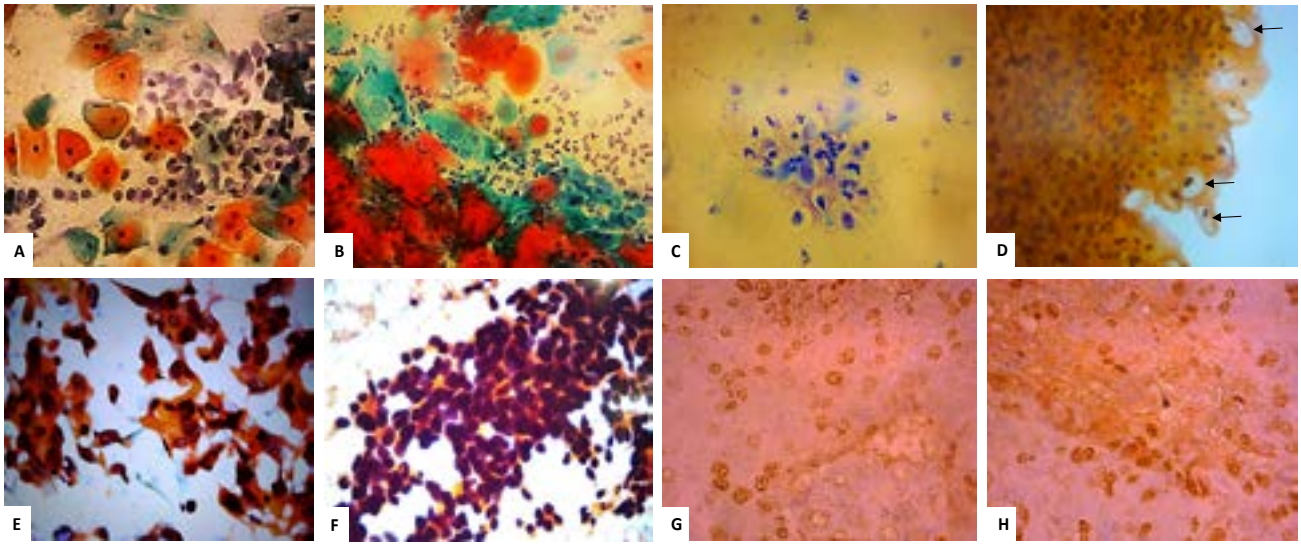

Figure 1: Photomicrographs of stained normal and abnormal cervical smears and abnormal tissue sections

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% Cl: 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% Cl: 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 ≤ 18 years, these participants surprisingly had lower prevalence of SIL than those who had their sex debut at ≥18years. The HIV+ women with sex debut at ≤ 21 years had higher prevalencef HSV-2 than their HIV- counterparts. The HIV+ debutants at ≤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% Cl: 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
				HIV+	HIV-		HIV+	HIV-		HIV+	HIV-		HIV+	HIV-	
		N= 105	N= 105	n= 34	n= 14		n= 43	n= 28		n= 44	n= 28		n= 79	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)	**0.008**
	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)	**0.021**	23 (74.2)	17 (40.5)	**0.009**
	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)	**0.050**	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)	**0.017**	28 (38.9)	13 (28.3)	0.836	30 (41.7)	6 (13.0)	**0.001**	50 (69.4)	19 (41.3)	**0.004**
	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)	**0.010**
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)	**0.018**	21 (40.4)	10 (35.7)	0.811	25 (48.1)	3 (10.7)	**0.001**	38 (73.1)	12 (42.9)	**0.015**
	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)	**0.003**
Tribe	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)	**0.008**	77 (78.6)	25 (46.3)	**0.000**
	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
	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
Education	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)	**0.012**	12 (38.7)	10 (21.7)	0.128	16 (51.6)	10 (21.7)	**0.013**	23 (74.2)	21 (45.7)	**0.019**
	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
Residence	Urban	100(95.2)	64 (61.0)	32 (32.0)	9 (14.1)	**0.010**	41 (64.1)	19 (29.7)	0.184	40 (40.0)	15 (23.4)	**0.041**	76 (76.0)	32 (50.0)	**0.001**
	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
Religion	Christian	65 (61.9)	93 (88.6)	19 (29.2)	12(12.9)	**0.014**	29 (44.6)	22 (23.7)	**0.009**	25 (38.5)	25 (26.9)	0.164	48 (27.7)	40 (43.0)	**0.000**
	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)	**0.012**	31 (37.5)	8 (66.7)	0.466
Income Status	Low	77 (73.3)	34 (32.4)	25 (32.5)	4 (11.8)	**0.033**	35 (45.5)	7 (20.6)	**0.019**	34 (44.2)	11 (32.4)	0.297	59 (76.6)	16 (47.1)	**0.012**
	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)	**0.024**
	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
Parity	Null	46 (43.8)	39 (37.1)	16 (34.8)	6 (15.4)	**0.050**	20 (43.5)	7 (17.9)	**0.019**	18 (39.1)	12 (30.8)	0.498	33 (71.7)	17 (43.6)	**0.015**
	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
	Multiparous	53 (50.5)	58 (55.2)	16 (30.2)	6 (10.3)	**0.016**	22 (41.5)	17 (29.3)	0.233	22 (41.5)	15 (25.9)	0.107	43 (81.1)	25 (43.1)	**0.000**
Age at sex debut	≤18years	26 (24.8)	40 (38.1)	6 (23.1)	4 (10.0)	0.175	14 (53.8)	8 (20.0)	**0.007**	13 (50.0)	11 (27.5)	0.074	19 (73.1)	17 (42.5)	**0.023**
	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)	**0.013**	36 (75.0)	19 (44.2)	**0.005**
	≥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)	**0.005**	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)	**0.048**	23 (42.6)	23 (35.4)	0.454	38 (70.4)	30 (46.2)	**0.010**
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)	**0.015**	78 (75.0)	39 (43.8)	**0.000**
	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)	**0.002**	43 (41.0)	25 (27.8)	0.070	44 (41.9)	21 (23.3)	0.006	79 (75.2)	39 (43.3)	**0.000**
	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)	**0.013**	43 (42.6)	21 (29.6)	0.151	41 (40.6)	20 (28.2)	0.107	76 (75.2)	34 (47.9)	**0.000**
	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
				HIV+	HIV-		HIV+	HIV-		HIV+	HIV-		HIV+	HIV-	
		N= 105	N= 105	n= 34	n= 14		n= 43	n= 28		n= 44	n= 28		n= 79	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	HSV-2	EBV
		IgG	IgM	IgG	IgM	IgG	IgM	IgG+IgM	IgG+IgM	IgG+IgM
Cut-off value		**1.071**	**0.438**	**1.520**	**0.102**	**1.570**	**1.030**			
HIV Seropositive										
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)
HIV Seronegative										
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)
HIV+ (n=105)		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)
HIV- (n=105)		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)
HIV+ vs HIV- p-value		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[23] but lower than the 61.1% and 76% reported in HIV+ women at Tanzania and Zambia, respectively[11,24]. In this study, te prevalence of SIL is higher than the prevalence reported in Etiopian HIV+ women (13.6%) and HIV- women (5.2%), respectively[25]. Studies have shown that HIV infection increases the risk for CIN (SIL) by 2.3 to 5 times[26-28]. 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[29]. 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[29]. A recent study revealed that HSV-2 seropositive women have Ca risk of 2- to 9-fold[30]. The increasing 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% Cl: 1.31-5.96, 1.23-7.20 and 1.50-7.86), respectively[31].

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[20]. 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 of have been made in earlier studies[20-21]. 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[32,33]. This might explains 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[20,34,35]. 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[18]. 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[36].

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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