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Seroprevalence and Co-Infection of Human Papillomavirus (HPV) Genotypes Among HIV Positives

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Abstract: Human Papillomavirus (HPV) infection is now considered a sexually transmitted disease with particular types being highly oncogenic in nature. High-risk HPV virus infection is a cause of nearly all cases of cervical cancer amongst women. This study determined the seroprevalence of HPV type-16 and type-18 among HIV positive. Blood samples were processed using ELISA test kit and PCR technique. ELISA detected HPV antibodies in 1036(4.4%) of the samples out of which 14(13.6%) and 19(18.4%) were positive for HPV type-18 and type 18 using PCR. People in the age group 51-60 years had an HPV prevalence of 41(77.41%) while those with secondary education had a prevalence of 51(81.0%). Those who did not state the number of sexual partners they had was 92(76.7%). High levels of HPV antibodies were also found among civil servants (n = 52, 50.5%), traders (n = 29, 76.3%) and widows (n = 49, 72.1%). HPV infection was highest among individuals between 41-50 years with HPV type-16 being 9(34.6%) while HPV type-18 was 11(42.3%). HPV infection (type-16) was also recorded in rural dwellers (n = 11, 14.7%) and traders (n = 7, 24.1%). However, type -18 HPV infection among rural dwellers and traders was 22.7% (n = 17) and 37.9% n = 11) respectively. HPV type-16 (n = 11, 12%) and HPV type-18 (n = 16, 17.4%) was also recorded for those that did not state the number of sexual partners they had. Our results show the presence of HPV type-16 and type18 infection among the HIV-1 positive individuals. The results show that HPV is a co-infection in HIV-1 positive individuals. We conclude that some of the major risk factors associated with HPV infection among HIV-1 positive individuals are age and occupational status. There is need to include HPV screening as one of the vital tests during HIV-1 screening since early detection of HPV helps in reducing female mortality due to cervical cancer.

Keywords: Human Papillomavirus, HIV, Co-Morbidities, Viral Infection, Pathogenesis

Introduction

Human Papillomavirus (HPV) is a DNA-virus responsible for the most widespread Sexually Transmitted Disease (STD) in the world (Nyasenu *et al*., 2019; Tartaglia *et al*., 2017). HPVs are subdivided into low and high risk according to their oncogenic potential. High-risk oncogenic HPVs are often associated with a high-grade Intraepithelial Lesion (SIL) and cervical malignancies (Tartaglia *et al*., 2017). The relationship between cervical cancer and HPV infection is well established in the literature, with HPV detection in almost 100% of cases (Kobayashi *et al*., 2018). The high-risk human papillomavirus genotypes 16 and 18 together are contributed in the majority of all cervical carcinomas (70%) in the general human population

(Tartaglia *et al*., 2017; Wang and Palefsky, 2015). The ability of the virus to persist in the host may be increased by factors that lead to suppression or abolition of cellular immunity, such as the use of cytotoxic drugs in transplant recipients, innate or acquired immunodeficiency such as that caused by the Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS) (Wakabayashi *et al*., 2018; Marchetti *et al*., 2013; Mombelli *et al*., 2015). According to data from the United Nations Program on HIV/AIDS (UNAIDS), there are 36.9 million people in the world living with HIV/AIDS and women already represent almost half of infected adults (Mombelli *et al*., 2015; UNAIDS, 2015). In women with HIV/AIDS, the prevalence of HPV infection is higher; it increases with decreasing immunity and tends to be more persistent and more at risk of developing Cervical Intraepithelial Neoplasia (CIN) and cervical cancer (Wang and Palefsky, 2015; Marchetti *et al*., 2013). Previous studies have shown that HIV-1 positive women are 2 to 12 times more likely to develop malignant cervical cancers than HIV-1 negative women (Mapanga *et al*., 2018). According to available statistics, Nigeria has the fifth highest incidence rank in terms of death count from cervical cancer, after India, China, Brazil and Bangladesh with a national mortality rate of 250/100,000 women (Bisi-Onyemaechi *et al*., 2018; Olubodun *et al*., 2019). This shows that the mortality rate from cervical cancer in Nigeria is very alarming and demands urgent health policy, research and development and proper screening and treatment measures to contain the emergence and spread of this oncogenic viral infection or disease (i.e., cervical cancer mediated by HPV). Available information indicates that the prevalence rate of HIV-1 in Ebonyi state, South-Eastern Nigeria was as high as 4.6% (Okoye *et al*., 2012). This implied that the number of women who formed at least half of them and whose risk of cervical cancer is further heightened by the co-morbidity of HIV-1 and HPV is also expanding. Unfortunately, despite the fact that HPV can be detected by simple screening tests and if treated early does not progresses to cause cervical cancer, minimal efforts are being made by both corporate and government agencies to encourage young women at risk to be screened for HPV infection. With this sinister health situation which is actually worsening, it is very needful that efforts should be made to salvage the female folk from this ravaging cervical cancer known to be caused by HPV high-risk genotypes 16 and 18 (Tartaglia *et al*., 2017; Kobayashi *et al*., 2018; Wakabayashi *et al*., 2019). Given these considerations, this pilot study aimed to screen the HPV seroprevalence among HIV-1 infected women using Enzyme-Linked Immunosorbent Assay (ELISA) and PCR techniques to detect HPV high-risk genotypes 16 and 18 in blood samples of HIV-1 positive and negative women groups, making a comparative study between the two groups in Ebonyi state, south-eastern Nigeria.

Materials and Methods

Ethical Approval

This study was approved by the local ethics committee of Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria.

Study Population

This study was carried out after obtaining informed consent from all participants. Blood samples were collected from 160 randomly selected females (18-65 years) referred to a tertiary hospital in the South-Eastern part of Nigeria. The inclusion criteria included females who tested positive for HIV-1 as well as those who tested negative for the virus. Those excluded from the study included males, children, females who have a menstrual cycle and pregnant women.

Sample Collection

5 mL of venous blood samples were aseptically collected from each study participant into EDTA bottles using a disposable sterile safety vacutainer syringe with the needle through venepuncture. Each specimen was singly labelled and transported to the microbiology laboratory in cold box with frozen ice packs to achieve condition of about 4-8°C. 2 mL of whole blood sample was used for the PCR analysis while the remaining 3 mL of blood was centrifuged at 4000 rpm for 5 min to obtain serum for screening of specific anti-HPV IgG antibodies using ELISA technique. Serum was transferred into singly labelled sterile cryovials and stored at -20°C until use (Nyasenu *et al*., 2019; Marchetti *et al*., 2013).

Detection of HPV Using ELISA

This was carried out using ELISA test kit (MBS284245 and MBS284267 product of MyBiosource, USA) according to the manufacturer's instructions. The microtiter plate provided in the kit was pre-coated with an antigen-specific to HPV IgG antibody. The ELISA analytical technique of the kit is based on HPV-IgG antibody-HPV-IgG antigen targets in samples used for quantitative analysis of the presence of Human papillomavirus antibody IgG (HPV-IgG) in the human sample.

DNA Extraction and Molecular Analysis

DNA extraction from whole blood samples was done according to manufacturer's instruction using Viral Zymogen Kit (Zymo Research Corporation, USA) and kept in -20°C until use. PCR technique was carried out according to the method of Fontaine *et al*. (2007) in a thermal cycler (Lumex instruments, Canada) for the detection of HPV genotypes 16 and 18 using specific primers (Table 1).

Table 1: Primers for HPV genotypes 16 and 18

Primer name	Primer sequence	Tm	Amplicon size
HPV-16 F	5′-TTTGGTCTACAACCTCCCCCAGGA-3′	66.28	105
HPV-16 R	5′-TTCTTTAGGTGCTGGAGGTGTATG-3′	62.86	105
HPV-18F	5′-CCTTGGACGTAAATTTTTGG-3′	56.30	115
HPV-18R	5′-CACGCACACGCTTGGCAGGT-3′	60.40	115

PCR amplification was performed in a 25 µL volume which consisted of 2.0 µL of 100 ng DNA, 2.5 µL of 10 X Buffer, 1.5 µL of 50 mM $MgCl_2$, 2.0 µL of 2.5 mMdNTPs, 0.2 µL of 500U DNA Taq polymerase, 1.0 µL of 10 µM of reverse and forward primers for HPV-16 or HPV-18 and 14.8 µ of 500 mL DEPC-treated water (Invitrogen Corporation, USA). The PCR cycling profile consisted of an initial step at 94°C for 5 min, 35 cycles of 94°C for 20 s, 60°C for 30 s, 72°C for 1 min and a 10-minute final extension at 72°C. Finally, 8 µL of the PCR products were electrophoresed in a 1.5% agarose gel containing 0.5 mg/mL ethidium bromide and photographed on Transilluminator UV light (Fotodyne Incorporated, Analyst Express, Japan).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS, Chicago, IL, USA). Simple frequency analyses, Fisher's exact test and Chi-square tests were used to compare two groups. *P*-values 0.05 were considered significant.

Results

The result of the seroprevalence of Human Papillomavirus (HPV) among the HIV positive and negative individuals is shown in Table 2. A total of 160 whole blood samples were analyzed for the presence of HPV specific antibodies. 103 blood samples analyzed with ELISA were seropositive to HPV IgG representing (64.4%) prevalence rate. Eighty-eight (85.4%) of the HIV-positive individuals were positive in the ELISA while 15(14.6%) of the non-HIV-positive were positive to ELISA. HPV infection was significantly detected in HIV-positive individuals (n = 88; 85.4%) compared to those who were HIV-negative ($\chi^2 = 64.89$, $P = 0.00001$).

Table 3 shows the prevalence of HPV according to age using ELISA test. Detection of HPV in individuals comprises of 5(20.8%) individuals of 21-30 years, 31(62%) individuals of 31-40 years, 26(86.7%) individuals of 41-50 years, 41(77.4%) individuals of 51-60 and none in individuals in other year group. More infection were found within the age group of 51-60 years 41(77.4%) and 31-40 31(62%) years of individuals. The result were statistically significant ($\chi^2 = 33.15$, $P = 0.0001$).

Table 4 shows the detection of Human papillomavirus among various age groups using PCR. HPV type-16 infections were detected in 2(6.5%) individuals of 31-40 years, 9(34.6%) individuals of 41-50 years, 3(97.3%) individuals of 51-60 years and none were found in the other age groups. More infection was found within the age group of 41-50 years 9(34.6%) for the HPV type-16 using specific primers (HPV-16 primer) ($\chi^2 = 13.40$, $P = 0.01$).

Then, HPV type-18 were detected in 1(3.2%) individuals of 31-40 years, 11(42.3%) individuals of 41-50 years, 7(17.1%) individuals of 51-60 years and none for the other age groups. More infection was found within the age group of 41-50 years 9(34.6%) and 11(42.3%) respectively for the HPV type-18 using specific primers (HPV-18). The result was statistically significant ($\chi^2 = 14.2$, $P = 0.003$).

Table 5 shows seroprevalence of Human papillomavirus according to the individual's occupation as detected with ELISA test. HPV was detected in 52(50.5%) individuals that was civil servants, 29(76.3%) individuals were traders, 14(53.8%) were artisans, 8(72.7%) individuals were farmers. Other occupational groups tested negative on the ELISA test. Civil servants having the highest prevalence of 52(50.2%) followed by traders having 29(76.3%) positive individuals. The result was statistically significant ($\chi^2 = 46.10$, $P = 0.00001$).

Table 6 shows the occupational distribution of HPV among the individuals as revealed in the PCR Test. HPV type-16 was detected in 14(13.6%) individuals. 5(9.6%) of the individuals tested positive were civil servants, 7(24.1%) were traders, 2(14.3%) were artisans. Other occupational groups were negative. Traders had the highest prevalence of 7(24.1%) positive individuals. Some of the farmers under the study tested positive to the screening done with ELISA test but tested negative to the PCR meaning that they have no high-risk HPV infection. There is no statistically significant difference in the result ($\chi^2 = 5.10$, $P = 0.28$). HPV type-18 was detected in 19(18.4%) individuals, 6(11.5%) of the individuals tested positive were civil servants, 11(37.9%) were traders, 2(14.3%) were artisans and none of the other occupational groups were positive with traders having the highest prevalence of 11(37.9%) positive individuals. Some of the farmers under the study tested positive to the screening done with ELISA test but tested negative to the PCR meaning that the HPV infection presence is of low-risk type. There was a statistically significant difference in the result ($\chi^2 = 10.10$, $P = 0.04$).

Table 2: Seroprevalence of HPV among the HIV positive and negative individuals using ELISA test

Samples source	Number examined n(%)	HPV positive n(%)	HPV negative n(%)
HIV-positive	100(62.5%)	88(85.4%)	12(21.1%)
HIV-negative	60(37.5)	15(14.6%)	45(78.9%)
Total	160(100)	103(64.4%)	57(35.6%)

($\chi^2 = 64.89$, $P = 0.00001$)

Table 3: Prevalence of Human papillomavirus in various age groups using ELISA test

Age (years)	Number examined n(%)	Number positive n(%) HPV	Number negative n(%) HPV
≤20	3(1.9)	0.00	3(100)
21-30	24(15)	5(20.8)	19(79.2)
31-40	50(31.2)	31(62)	19(38)
41-50	30(18.8)	26(86.7)	4(13.3)
51-60	53(33.1)	41(77.4)	12(22.6)
Total	160(100)	103(64.4)	57(35.6)

($\chi^2 = 33.15$, $P = 0.0001$)

Table 4: Detection of HPV among various age groups using PCR

Age (YRS)	Number examined	Number positive (%) HPV-16	Number negative (%) HPV-16	Number positive (%) HPV-18	Number negative (%) HPV-18
21-30	5(20.8)	0(00)	5(100)	0.00	5(100)
31-40	31(62)	2(6.5)	29(93.5)	1(3.2)	30(96.8)
41-50	26(86.7)	9(34.6)	17(65.4)	11(42.3)	15(57.7)
51-60	41(77.4)	3(7.3)	38(92.7)	7(17.1)	34(82.9)
Total	103(64.4)	14(13.6)	89(86.4)	19(18.4)	84(81.6)

($\chi^2 = 13.40$, $P = 0.01$) ($\chi^2 = 14.2$, $P = 0.003$)

Table 5: Seroprevalence of HPV according to the individual's occupation as detected with ELISA test

Occupation	Number examined (%)	Number positive (%) HPV	Number negative (%) HPV
Civil servants	063(39.4)	052(50.5)	11(17.5)
Students	020(12.5)	000.00.0	20(100)
Traders	038(23.7)	029(76.3)	09(23.7)
Artisans	026(16.2)	014(53.8)	12(46.2)
Farmers	011(6.9)	008(72.7)	03(27.3)
None	002(1.3)	000.00.0	02(100)
Total	160(100)	103(64.4)	57(35.6)

($\chi^2 = 46.10$, $P = 0.00001$).

Table 6: Occupational Distribution of HPV among the individuals as revealed in PCR Test

Occupation	Number examined (%)	Number positive (%) HPV-16	Number negative (%) HPV-16	Number positive (%) HPV-18	Number negative (%) HPV-18
Civil servants	52(50.5)	5(9.6)	47(90.4)	6(11.5)	46(88.5)
Students	0.00	0.00	0.00	0.00	0.00
Traders	29(76.3)	7(24.1)	22(75.9)	11(37.9)	18(62.1)
Artisans	14(53.8)	2(14.3)	12(85.7)	2(14.3)	12(85.7)
Farmers	8(72.7)	0.00	8(100)	0.00	8(100)
Total	103(64.4)	14(13.6)	89(86.4)	19(18.4)	84(81.6)

($\chi^2 = 5.10$, $P = 0.28$) ($\chi^2 = 10.10$, $P = 0.04$)

Table 7 shows the spread of HPV according to the individual's marital status using ELISA test. HPV infections were in a total number of 103(64.4%) of the total individuals using ELISA and 40(90.9%) were married, 14(63.6%) were divorced and 49(72.1%) were widows. More infection was found in the widows 49(72.1%) followed by the married 40(90.9%) with the widows have the highest prevalence of HPV using ELISA test. There was statistically significant difference in the result ($\chi^2 = 57.23$, $P = 0.00001$)

Table 8 shows the seroprevalence of HPV among the individuals according to their marital status using PCR. Some of the individuals that were tested positive using ELISA test were also positive using PCR, 3(21.4%) were divorced and 11(22.4%) were widows. More infections were found among the widows 11(22.4%) using PCR with specific primers to HPV type-16 infections There is a statistically significant difference in the result ($\chi^2 = 9.53$, $P = 0.02$).

Using HPV type-18 primers, some of the individuals that were tested positive using ELISA test were also positive using PCR, 2(14.3%) were divorced and 17(32.7%) were widows. More infection was found in the widows 17(34.7%) using PCR with specific primers to HPV type-18 infection. There was a statistically significant difference in the result (χ^2 = 16.04, P = 0.001).

Table 9 shows the seroprevalence of HPV according to their educational status using ELISA test. Out of the 103 (64.4%) positive individuals, 15(53.6%) were uneducated, 16(43.2%) had primary education, 51(81.0%) secondary education and 21(65.6%) had tertiary education. Infections were more in individuals with secondary education 51(81.0%) then followed by tertiary level with 21(65.6%). There was a statistically significant difference in the result (χ^2 = 16.20, P = 0.001).

Table 10 shows the seroprevalence of HPV according to their educational background using PCR with specific primers to HPV type-16 and type-18, 3(20.0%) were uneducated, 7(43.8%) had primary education, 3(5.9%) secondary education and 1(4.8%) had tertiary education. More infections were found among the individuals at the primary level 7(43.8%) in HPV type-16 using specific primers. There is a statistically significant difference in the result (χ^2 = 16.96, P = 0.0007). Then, using PCR with specific primers to HPV type-18, 4(26.7%) were uneducated, 9(56.2%) had primary education, 5(9.8%) had secondary education and 2(9.5%) had tertiary education. More infections were found in individuals with primary education 9(56.2%) in HPVtype-18 using specific primers. There was a statistically significant difference in the result (χ^2 = 18.70, P = 0.0003).

Table 11 shows seroprevalence of HPV according to place of residence using ELISA test. A total number of 160 individuals were examined using ELISA Test, 63(39.4%) of the individuals were residing in an urban area and the other 97(60.6%) of the individuals resides in a rural area. Out of the 103(64.4%) positive individuals, 28(44.4%) were residing in an urban area whereas the 75(77.3%) positive individuals resides in a rural area. There was more infection found among the rural dwellers 75(77.3%). There was statistically significant difference in the result (χ^2 = 18.0, P = 0.00002).

Table 12 shows the seroprevalence of HPV according to place of residence using PCR with specific primers to the high risk types. out of the 103(64.4%) positive individuals to ELISA test, 28(44.4%) were urban dwellers and 75(77.3%) were rural dwellers. 33(10.7%) of the urban dwellers tested positive to HPV using HPV type-16 specific primers and 11(14.7%) of the rural dwellers tested positive to HPV type-16 specifc primers with the rural dwellers having more infections. There was no statistically significant difference in the result (χ^2 = 0.27, P = 0.60).

Again, using PCR with specific primers to HPV type-18. Out of the 103(64.4%) positive individuals to ELISA test kit, 28(44.4%) were urban dwellers and 75(77.3%) were rural dwellers. 2(7.1%) of the urban dwellers tested positive using HPV type-18 specific primers and 17(22.7%) of the rural dwellers tested positive using the same primers with the rural dwellers having more infection 17(22.7%). There was no statistically significant difference in the result (χ^2 = 3.30, P = 0.07).

Table 13 shows seroprevalence of HPV according to the number of sexual partners they had using ELISA test. Out of the 103(64.4%) individuals that were tested positive to ELISA test, 40(25%) had less than five partners and the rest did not state the numbers of sexual partners they had. 11(27.5%) of the individuals with less than five sexual partners were positive using ELISA test kit and 92(76.7%) of those that did not state the number of sexual partners they had tested positive. The result was significant stastically (χ^2 = 31.62, P = 0.0001).

Table 14 shows seroprevalence of HPV according to the number of sexual partners they had using PCR with specific primers, out of the 103(64.4%) individuals that tested positive using ELISA test, 3(27.3%) of the individuals that had less than five sexual partners was positive to HPV type-16 specific primers and 11(12%) of those individuals that did not state the number of partners they had were to HPV type-16 specific primers. More infection was found among those that did not state the number of sexual partners they had 11(12 %). There were no statistically significant difference in the result (χ^2 = 1.96, P = 0.16). Using PCR with specific primers to HPV type-18, 3(27.3%) of the individuals that had less than five sexual partners were positive to HPV type-18 specific primers and 16(17.4%) of those individuals that did not state the number of partners they had, was to HPV type-18 specific primers. More infection was found among those that did not state the number of sexual partners they had 16(17.4%). There was no statistically significant difference in the result (χ^2 = 0.64, P = 0.42).

Table 7: Spread of HPV according to the individual's marital status using ELISA test

Marital status	Number examined (%)	Number positive (%) HPV	Number negative (%) HPV
Married	44(27.5)	040(90.9)	04(9.1)
Single	26(16.2)	000.00.0	26(100)
Divorce	22(13.8)	014(63.6)	08(36.4)
Widows	68(42.5)	049(72.1)	19(27.9)
Total	160(100)	103(64.4)	57(35.6)

(χ2 = 57.23, P = 0.00001)

Table 8: Prevalence of HPV among the individuals according to their marital status using PCR

Marital Status	Number examined (%)	Number positive (%) HPV-16	Number negative (%) HPV-16	Number positive (%) HPV-18	Number negative (%) HPV-18
Married	040(90.9)	00.0000	40(100)	00.0000	40(100)
Single	000.00.00	00.0000	00.0000	00.0000	00.00.0
Divorce	014(63.6)	03(21.4)	11(78.6)	02(14.3)	12(85.7)
Widows	049(72.1)	11(22.4)	38(77.6)	17(34.7)	32(65.3)
Total	103(64.4)	14(13.6)	89(86.4)	19(18.4)	84(81.6)

($\chi^2 = 9.53$, $P = 0.02$) ($\chi^2 = 16.04$, $P = 0.001$)

Table 9: Seroprevalence of HPV according to their educational status using ELISA test

Educational status	Number examined (%)	Number positive (%) HPV	Number negative (%) HPV
Uneducated	28(17.5)	15(53.6)	13(46.4)
Primary	37(23.1)	16(43.2)	21(56.8)
Secondary	63(39.4)	51(81.0)	12(19.0)
Tertiary	32(20.0)	21(65.6)	11(34.4)
Total	160(100)	103(64.4)	57(35.6)

($\chi^2 = 16.20$, $P = 0.001$)

Table 10: Prevalence of HPV according to their educational background using PCR

Educational status	Number examined (%)	Number positive (%) HPV-16	Number negative (%) HPV-16	Number positive (%) HPV-18	Number negative (%) HPV-18
Uneducated	15(53.6)	3(20.0)	12(80.0)	4(26.7)	11(73.3)
Primary	16(43.2)	7(43.8)	9(56.2)	9(56.2)	7(43.8)
Secondary	51(81.0)	3(5.9)	48(94.1)	5(9.8)	46(90.2)
Tertiary	21(65.6)	1(4.8)	20(95.2)	2(9.5)	19(90.5)
Total	103(64.4)	14(13.6)	89(86.4)	19(18.4)	84(81.6)

($\chi^2 = 16.96$, $P = 0.0007$) ($\chi^2 = 18.70$, $P = 0.0003$)

Table 11: Seroprevalence of Human papillomavirus according to place of residence using ELISA test

Place of residence	Number examined (%)	Number positive (%) HPV	Number negative (%) HPV
Urban	63(39.4)	28(44.4)	35(55.6)
Rural	97(60.6)	75(77.3)	22(22.7)
Total	160(100)	103(64.4)	57(35.6)

($\chi^2 = 18.0$, $P = 0.00002$)

Table 12: Shows the Prevalence of HPV according to place of residence using PCR

Place of Residence	Number examined (%)	Number positive (%) HPV-16	Number negative (%) HPV-16	Number positive (%) HPV-18	Number negative (%) HPV-18
Urban	28(44.4)	3(10.7)	25(89.3)	2(7.1)	26(92.9)
Rural	75(77.3)	11(14.7)	64(85.3)	17(22.7)	58(77.3)
Total	103(64.4)	14(13.6)	89(86.4)	19(18.4)	84(81.6)

($\chi^2 = 0.27$, $P = 0.60$) ($\chi^2 = 3.30$, $P = 0.07$)

Table 13: Seroprevalence of Human papillomavirus according to number of sexual partners using ELISA test

Sexual partners	Number examined (%)	Number positive (%) HPV	Number negative (%) HPV
<5	40(25)	11(27.5)	29(72.5)
5-10	0.00	0.00	0.00
>10	0.00	0.00	0.00
Not stated	120(75)	92(76.7)	28(23.3)
Total	160(100)	103(64.4)	57(35.6

($\chi^2 = 31.62$, $P = 0.0001$)

Table 14: Prevalence of HPV according to the number of sexual partners they had using PCR

Sexual partners	Number examined (%)	Number positive (%) HPV-16	Number negative (%) HPV-16	Number positive (%) HPV-18	Number negative (%) HPV-18
<5	11(10.7)	03(27.3)	8(72.7)	3(27.3)	8(72.7)
5-10	0.00	0.00	0.00	0.00	0.00
>10	0.00	0.00	0.00	0.00	0.00
Not stated	92(89.3)	11(12)	81(88)	16(17.4)	76(82.6)
Total	103(64.4)	14(13.6)	89(86.4)	19(18.4)	84(81.6)

($\chi^2 = 1.96$, $P = 0.16$) ($\chi^2 = 0.64$, $P = 0.42$)

Discussion

This study evaluated the seroprevalence of Human Papillomavirus (HPV) IgG antibodies from clinical samples collected from 100 HIV-1 positive and 60 HIV-1 negative individuals attending HIV clinic in a tertiary hospital in the south-eastern part of Nigeria. The results obtained with the ELISA test that detected the high prevalence rate (64.4%) of the presence of specific IgG to HPV established the association of HPV as a common infection in HIV positive individuals in this part of the world which are mainly the low-risk types. The result is in agreement with the study carried out by Ngwu and Ezeifeka (2015) in which a similar rate of HPV was detected in their study. The seroprevalence of HPV according to age using ELISA test shows that individuals between the age group of 51-60 years 41(77.4%) and 31-40 years 31(62%) years have a higher prevalence rate. This is in agreement with a recent study in South African in which a prevalence rate of 61.8% was reported among adult women (Dianne *et al*., 2008). The study conducted in Central and South America, Mozambique, Senegal, South Africa and in women in other parts of Nigeria also reported a high prevalence of HPV-16 and HPV-18 with an increase in the age of the participants as reported in this study (Dianne *et al*., 2008; Smith *et al*., 2008; Castellsague *et al*., 2001; Xi *et al*., 2003; Kuhn *et al*., 2000; Clarke *et al*., 2011; Thomas *et al*., 2004). In a study of cervical cancer cases from Lagos in south-south Nigeria, HPV type-16 and type-18 were present in 65.2 of the study participants (Abdulkareem, 2016).

The possible cause may be due to cell degeneration as one age, especially in females. Although, in Africa, the age-specific prevalence of human papillomavirus differs across countries. Human papillomavirus prevalence was highest in younger women and decreased steadily with age in Kenya, Uganda and Zimbabwe but generally reached a plateau at approximately 40 years of age in urban Nigeria and Mozambique. Whereas in Senegal, South Africa and another study from rural Nigeria, the peak prevalence in young women was accompanied by a second but smaller increase in prevalence among older women. In the Gambia, HPV prevalence remained relatively constant among women aged 15 to 54 years. Possible reasons include variations in sexual behavioral practices, sexual hygiene, co-morbidities, polygamy, male sexual partner behaviour and male circumcision. However, there was a slight difference in another study done in Abuja, the Federal Capital Territory where it was noted that the age range with the highest positivity of antibodies was among the young age group.

Among the HIV infected respondents, there was a significant difference in the positivity of the antibodies by ELISA and on PCR test compared to HIV uninfected respondents. This is in consonance to what should ordinarily be expected considering that immune competence plays a significant role in determining the vulnerability of an individual to an infective agent. HIV destroys the immune system of an individual by attacking, destroying and hence reducing the number of the $CD4^+$ T cells. These $CD4^+$ cells play a central prominent role in the activation and mobilization of immunoactive agents - cells and chemicals- to fight and protect the body against invading pathogens. Therefore, when they are deficient (commonly called low CD4 count), the patient is prone to infective agents and cancers. Moreover, HIV infection being a sexually transmitted infection, it follows that other sexually transmitted infections such as HPV should also be commoner among HIV infected individuals. Also, it is common knowledge that apart from the sexual route, there are many other means of transmitting HIV such as by shearing of sharps, transfusion of improperly screened blood and blood products etc. This study agrees with a similar study done at Ibadan, Nigeria which also noted significant difference in prevalence of HPV among HIV positive and HIV negative individuals (Ngwu and Ezeifeka, 2015); and also with the study among West African immigrants resident in Southern Italy where the prevalence was noted to be more among HIV positive individuals (Maria *et al*., 2007).

Marital status is another socio-demographic factor that significantly affects the predisposition to acquiring HPV infection. In this study, the married and widowed were significantly more affected by HPV compared to the unmarried. Since HPV is sexually transmitted and it has been noted that the more the number of sexual partners a woman had in her lifetime the more the chances of getting infected with HPV; it follows that the married and widowed may have had numerous sexual exposures that predispose them to the risk of getting the virus. The risk is further heightened by the high salt of promiscuity and marital infidelity among the male folk in our society where promiscuity among the male gender is trivialized by male chauvinistic African traditions. The problem in this situation is that even when married women keep themselves within marital sanctity, their husbands may still expose them to the risks of sexually transmitted infections including HPV. Regarding the fact that being widowed was considered predisposing for HIV-seropositivity, a study performed in an outpatient clinic of a reference center in the STI area, located in São Paulo, which evaluated sexuality and reproductive health of women living with HIV/AIDS, claimed that this data was to be expected since many of these women became widows because their partners had AIDS (Santos *et al*., 2002; Sauvaget *et al*., 2011). And HPV infection is being transmitted through the same route as HIV (Joice *et al*., 2015; Chukwuali *et al*., 2003).

Education did not have any significant effect on the seropositivity of the respondents to HPV. This agrees with a previous study on the impact of education on lifestyle modification which noted that education had no significant impact on the sexual behaviours of women. Though highly educated women may be more classy and choosy in terms of whom to relate sexually with, it is worthy of note that even the rich men of a high social class who are promiscuous can still be carriers of sexually transmitted infections, including HPV.

Conclusively, some of the major risk factors associated with HPV infection among HIV-1 positive individuals in this study are age and occupational status. And HPV screening should be included as one of the vital tests during HIV-1 screening to help mitigate the morbidities and/or mortalities associated with cervical cancer in women.

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Author's Contributions

Okoro Nworie: Drafted manuscript for intellectual content and supervised the study.

Euslar Onu: Conducted the experiment and analyzed results.

Ogbonnaya Ogbu: Revised final version for submission, supervised the study and analyzed results; **Chika Ejikeugwu** wrote the manuscript, designed and interpreted the data.

Maduka Agah: Drafted manuscript for intellectual content and analyzed results.

Uchenna Ekuma: Analyzed the results and conducted part of the experiment.

Emmanuel Onu: Analysed and interpreted the data.

Ifeanyichukwu Iroha: Finalized and approved the final draft before submission.

Ethics

Approval for the study was obtained from the ethical committee of AEFETHA; and oral and written consents for the participation of the patients in the study were obtained from the patients.

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