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Surveillance of High-Risk Type-16 and 18 Human Papillomavirus among HIV Positive and Non-Positive Individuals at Federal Teaching Hospital Ebonyi State Nigeria

Euslar Nnenna Onu[1*], Donatus Chukwuma Ilang[1], Cornelius Ituma Azi[2], Theophilus Onwe Eze[3], Chinedu Obasi Akpa[4], Okechukwu Emmanuel Ogbonna[5], Samuel Eleojo Agada[6], Michael Chinedu Onwe[7]

[1]Department of Microbiology, Faculty of Biological Sciences, Alex-Ekwueme Federal University Ndufu-Alike, Ikwo, Nigeria
[2]Northern Care Alliance NHS Foundation Trust, Manchester, United Kingdom
[3]Park Hospital Burntstump Country Park, Sherwood Lodge Drive, Nottingham, United Kingdom
[4]Department of Haematology, Faculty of Basic Clinical Sciences, Alex-Ekwueme Federal University Ndufu-Alike Ikwo, Abakaliki, Nigeria
[5]Department of Stems Cell and Regenerative Medicine, University of Chester, Chester, United Kingdom
[6]Department of Biotechnology, University of Chester, Chester, United Kingdom
[7]Birmingham, West Midlands, United Kingdom
Email: *euslar.onu@funai.edu.ng

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Abstract

This study was carried out to determine the seroprevalence of high risk human papillomavirus type-16 and type-18 among people living with HIV and individuals who are seronegative to HIV infection. A total of 400 Blood samples were collected from 200 persons living with HIV and 200 negative volunteers were analysed using the Enzyme Linked Immunosorbent Assay test. The positive samples were subjected to further tests using a Polymerase chain reaction with specific primers for human papillomavirus type-16 and type-18. Using the ELISA test, Human papillomavirus antibodies were detected in 180 (45%) of the samples, out of which 19 (10.6%) and 17 (9.4%) were positive using PCR with human papillomavirus type-16 and type-18 primers respectively. Furthermore, using the ELISA test, Human papillomavirus antibodies were highest among people living with HIV, age group 51 - 60 years 85 (47.2%), hotelier 73 (64.6%) and those living in the Urban 147 (47%). Traders were also found to have high level of human papillomavirus antibodies 41 (50.6%), while out of the 231 (57.7%) of the individuals that are married, 106 (45.9%) of them had human papillomavirus antibodies. Using PCR with HPV type-16 and HPV type-18 specific primers, the infection was found to be highest among

individuals between 51 - 60 years with HPV type-16 being 69 (81.2%) while HPV type-18 was highest among the age group 41 - 50 having 11 (20%). This work shows that there is a need to include human papillomavirus screening as one of the vital tests since early detection of the presence of the virus helps in the reduction of the female mortality rate due to cervical cancer.

Keywords

Human Papillomavirus, Mortality, Antibodies, Primers, Cervix, Cancer

1. Introduction

Most Human Papillomavirus (HPV) infections are subclinical with no physical symptoms [1]. However, in some people subclinical infections may become clinical and cause benign papillomas such as warts or squamous cell papilloma [2]. Usually, premalignant lesions do progress to cancers of the cervix, vulva, vagina, penis, oropharynx and anus [3]. However, HPV type-16 and HPV type-18 are particularly known to cause about 80% of cervical cancer cases across the world [4]. The virus can be transmitted through direct contact such as sexual intercourse, and vertically from mother to child during delivery. High-risk Human papillomavirus infection is a cause of nearly all cases of cervical cancer [5].

Cancer of the cervix uterine is most common in females. Worldwide about 500 000 women acquire the disease annually and about 75% are from developing countries, while 300,000 women die of the disease annually [6]. Human papillomavirus infection rates are higher in developing regions (42.2%) than in developed regions (22.6%). Nevertheless, the prevalence is quite high in Eastern Europe (21.4%) and low in North Africa (9.2%) and Western Asia (2.2%), regardless of development [7].

Human papillomavirus infection is considered a sexually transmitted disease with particular types being highly oncogenic. The WHO's International Agency for Research on Cancer (IARC) has classified HPV into three groups: "carcinogenic HPV types, type-16 and type-18; probably carcinogenic HPV types 31 and 33 and possibly carcinogenic other HPV types except 6 and 11" [8]. The diagnosis of genital Human papillomavirus (HPV) infection has been done by indirect means known as cytology. Recently highly specific HPV DNA tests like Polymerase Chain Reaction tests have been developed for the detection of HPV in cervical/vaginal preparations [9]. These tests are very sensitive and contribute significantly to the early detection of the virus in the genital tract [10].

Screening for precursor lesions of the cervix has been in use for a long time in industrially advanced countries and it has led to a marked reduction in cervical cancer death rate by about 70% [11].

Human deficiency Virus (HIV) and the co-infection of HPV and HIV with attendant cervical cancer pose an additional cancer burden to HIV patients who are already at increased risk of various infections [12]. The management of HIV

infection poses an immense economic burden to both individuals and the government at all levels. The addition of the increased risk of cervical cancer which HPV generally confers leaves less to be desired by anybody [13].

In our society, it is a common experience that the incidence of cervical cancer is rather increasing [14]. This could be attributed to the increasing incidence of disease conditions that predispose women to cancers such as HIV and HPV despite the fact that HPV can be detected by simple screening tests and if treated early does not progress to cause cervical cancer.

According to available statistics, In Nigeria, cervical cancer is the third most common cancer and the second most frequent cause of cancer deaths among women aged between 15 and 44 years. In 2020, the latest year for which data is available, Nigeria recorded 12,000 new cases and **8,000 deaths** from cervical cancer [15]. This shows that the mortality rate from cervical cancer in Nigeria is very alarming [15].

Available information indicates that the prevalence rate of HIV in Ebonyi State was as high as 4.6 % [16]. This implied that the number of women whose risk of cervical cancer is further heightened by the co-morbidity of HIV and HPV is also expanding. With this sinister situation which is actually worsening by the day before us, it is very needful that efforts should be made to salvage the female folk from this ravaging cancer of the cervix known to be caused by HPV high-risk type-16 and type-18.

It is believed that sexually active females are prone to cervical infection with human papillomavirus and subsequently cancer and progress made in the treatment of cancer has always been linked to early detection [17]. It is hoped that this study will reveal the burden of HPV infection and the attendant risk of cancer of the cervix among adult females and HIV positive individuals in Abakaliki. It will also contribute to the solution of the ever-growing need for knowledge in the advancement needed in the better care for HIV positive individuals and the well-being of women.

2. Materials and Methods

2.1. Study Area

The research was carried out in Federal teaching Hospital located in the Abakaliki metropolis, the capital of Ebonyi state of Nigeria. Ebonyi state with an estimated population of about 4.3 million, lies between 7˚3'N Longitude 5˚4'E with a land mass approximated at 5932 square kilometres.

2.2. Ethical Approval

Approval for the study was obtained from the hospital's ethical committee, and consent was obtained from the patients for participation in the study.

2.3. Study Population

The study population includes a total of 400 individuals. 200 people living with

HIV and 200 People that are seronegative to the virus residing in Ebonyi State Nigeria and other female volunteers starting from January, 2023 to December, 2023 (see Table 1).

Table 1. The synthesised primers using a standard specific primers.

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′	56.3	115
HPV-18R	5′-CACGCACACGCTTGGCAGGT-3′	60.4	115

Source: Fontaine *et al.,* 2007.

2.4. Primers

The primers were synthesized by Inqaba Biotechnical Industries (pty) Ltd, South Africa using a standard specific primer [18].

2.5. Sample Analysis

All the participating individuals were tested for HIV to confirm their status before commencing the test which was performed according to the manufacturer's instruction. After that, the test was carried out using the Enzyme Linked Immuno-Sorbent Assay (ELISA) (MBS298245 & MBS298267 product of mybiosource U.S.A.) according to the manufacturer's instructions.

The polymerase chain reaction (PCR) was also done on the samples that were positive with the ELISA test for confirmation of the presence of Human papillomavirus high risk type-16 and type-18.

2.6. Statistical Analysis

The data was analysed using IBM SPSS statistic 25. A P-value less than 0.05 were considered statistically significant.

3. Results

Analysis of Results

A total of four hundred (400) blood samples were analysed for the presence of Human papillomavirus (HPV) specific antibodies. One hundred and eighty (180) blood samples analysed with Enzyme Linked Immuno-Sorbent Assay (ELISA) were seropositive to Human papillomavirus immunoglobulin G antibody, (HPV IgG) representing (45%) prevalence rate. One hundred and fifty-nine159 (88.3%) of the HIV-positive individuals were positive for the ELISA while 21 (11.7%) of the non-HIV-positive were positive for ELISA. All the one hundred and eighty samples that tested HPV positive using the ELISA test were subjected to a Polymerase chain reaction (PCR) test with specific primers. Using PCR with specific human papillomavirus type-16 and type-18 specific primers to detect the presence

of HPV type-16 and-18, both had a prevalence of 19 (10.6) and 17 (9.4) respectively.

Table 2 shows the Seroprevalence of HPV among the HIV positive and negative individuals using ELISA test. A total number of 200 (50%) samples were analysed from the HIV-positive individuals and a total number 200 (50%) samples were analysed from HIV-negative individuals. Human papillomavirus was detected in 180 (45%) of the total samples using the ELISA test, out of which 159 (88.3%) were from the HIV-positive samples and 21 (11.7%) were from HIV-negative samples. Detection of More infection was found among the HIV-positive individuals 159 (88.3%). The result was statistically significant ($\chi^2 = 192.364$, $P < 0.05$).

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

Sample Source	Examined n (%)	HPV		χ^2	P-value
		Positive n (%)	Negative n (%)		
HIV-Positive	200 (50.0)	159 (88.3)	41 (18.6)	192.364	<0.001
HIV-Negative	200 (50.0)	21 (11.7)	179 (81.4)		
Total	**400 (100.0)**	**180 (45.0)**	**220 (55.0)**		

($\chi^2 = 192.364$, $P < 0.05$).

Table 3 shows the prevalence of Human papillomavirus according to age using ELISA test. Human papillomavirus was detected in 180 (45%) individuals using the ELISA test. Detection of HPV in individuals comprises 7(3.9%) individuals of 20 - 30 years, 33 (18.3%) individuals of 31 - 40 years, 55 (30.6%) individuals of 41 - 50 years, 85 (47.2%) individuals of 51 - 60. More infections were found within the age group of 51 - 60 years 85 (47.2%) and 41 - 50 55 (30.6%) years of individuals. The result was statistically significant ($\chi^2 = 32.949$, $P < 0.05$).

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

Age (Years)	Examined n (%)	HPV		χ^2	P-value
		Positive n (%)	Negative n (%)		
20 - 30	44 (11.0)	7 (3.9)	37 (16.8)	32.949	<0.001
31 - 40	96 (24.0)	33 (18.3)	63 (28.6)		
41 - 50	86 (21.5)	55 (30.6)	31 (14.1)		
51 - 60	174 (43.5)	85 (47.2)	89 (40.5)		
Total	**400 (100.0)**	**180 (45.0)**	**220 (55.0)**		

($\chi^2 = 32.949$, $P < 0.05$).

Table 4 shows the detection of Human papillomavirus among various age groups using PCR. HPV was detected in 180 (45%) individuals using the ELISA test, and they were subjected to a Polymerase chain reaction (PCR) using HPV type-16 and type-18 primers. HPV type-16 infections were detected in 19 (10.6%).

3 (5.5%) individuals of 41 - 50 years, 16 (18.8%) individuals of 51 - 60 years. More infection was found within the age group of 51 - 60 years 16 (18.8%) for the HPV type-16 using specific primers (HPV-16 primer). The result is statistically significant ($\chi^2 = 12.391$, $P < 0.05$).

Then, HPV type-18 infections were detected in 17 (9.4%). HPV type-18 was detected in 11 (20%) individuals of 41 - 50 years, 7(8.2%) individuals of 51 - 60 years. More infections were found within the age group of 41 - 50 years 11 (20%) for the HPV type-18 using specific primers (HPV-18). The result was statistically significant ($\chi^2 = 10.850$, $P < 0.05$).

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

Age (yrs)	Examined n (%)	HPV-16			HPV-18		
		Positive n (%)	Negative n (%)	χ^2 (P-value)	Positive n (%)	Negative n (%)	χ^2 (P-value)
21 - 30	7 (3.9)	0 (0.0)	7 (100.0)	12.391* (0.006)	0 (0.0)	7 (100.0)	10.850* (0.013)
31 - 40	33 (18.3)	0 (0.0)	33 (100.0)		0 (0.0)	33 (96.8)	
41 - 50	55 (30.6)	3 (5.5)	52 (95.5)		11 (20.0)	44 (80.0)	
51 - 60	85 (47.2)	16 (18.8)	69 (81.2)		7 (8.2)	78 (92.8)	
Total	**180 (45.0)**	**19 (10.6)**	**161 (89.4)**		**17 (9.4)**	**163 (90.6)**	

($\chi^2 = 12.391$, $P < 0.05$) ($\chi^2 = 10.850$, $P < 0.05$).

Table 5 shows seroprevalence of Human papilllomavirus according to the individual's occupation as detected with ELISA test. HPV was detected in 23 (21.5%) individuals that were civil servants, 73 (64.6%) individuals were hoteliers, 41 (50.6%) were traders, 43 (43.4%) individuals were Artisans/skilled. Hoteliers having the highest prevalence of 73 (64.6%) followed by Artisans/Skilled having 43 (43.4%) positive individuals. The result was statistically significant ($\chi^2 = 42.558$, $P < 0.05$).

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

Occupation	Examined n (%)	HPV		χ^2	P-value
		Positive n (%)	Negative n (%)		
Civil servants	107 (26.7)	23 (21.5)	84 (78.5)	42.558	<0.001
Hotelier	113 (28.3)	73 (64.6)	40 (35.4)		
Traders	81 (20.3)	41 (50.6)	40 (49.4)		
Artisans/skilled	99 (24.7)	43 (43.4)	56 (56.6)		
Total	**400 (100.0)**	**180 (64.4)**	**220 (35.6)**		

($\chi^2 = 42.558$, $P < 0.05$).

Table 6 shows the occupational distribution of HPV among the individuals as revealed in the PCR Test. HPV type-16 was detected in 19 (10.6%) individuals. 17 (23.3%) of the individuals who tested positive were Hoteliers, 2 (2.4%) were

traders. Other occupational groups were negative. Hoteliers had the highest prevalence of 17 (23.3%) positive individuals. Some of the civil servants and Artisans under the study tested positive for the screening done with the ELISA test but tested negative for the PCR meaning that they had no high-risk HPV infection. There was statistically significant difference in the result ($\chi^2 = 21.723$, $P < 0.05$).

HPV type-18 was detected in 17 (9.4%) individuals, 17 (23.3%) of the individuals who tested positive were Hoteliers, and none of the other occupational groups were positive. There was a statistically significant difference in the result ($\chi^2 = 27.517$, $P < 0.05$).

Table 6. Occupational distribution of HPV among the individuals as revealed in PCR Test.

Occupation	Examined n (%)	HPV-16			HPV-18		
		Positive n (%)	Negative n (%)	χ^2 (P-value)	Positive n (%)	Negative n (%)	χ^2 (P-value)
Civil servants	23 (21.5)	0 (0.0)	23 (100.0)	21.723* (<0.001)	0 (0.0)	23 (100.0)	27.517* (<0.001)
Hotelier	73 (64.6)	17 (23.3)	56 (76.7)		17 (23.3)	56 (76.7)	
Traders	41 (50.6)	2 (2.4)	39 (95.1)		0 (0.0)	41 (100.0)	
Artisans	43 (43.4)	0 (0.0)	43 (100.0)		0 (0.0)	43 (100.0)	
Total	**180 (45.0)**	**19 (10.6)**	**161 (89.4)**		**17 (9.4)**	**163 (90.6)**	

($\chi^2 = 21.723$, $P < 0.05$) ($\chi^2 = 27.517$, $P < 0.05$).

Table 7 shows the spread of HPV according to the individual's marital status using the ELISA test. HPV infection was not detected among the single; however, it was detected in 106 (45.9%) that were married, 27 (71.1%) individuals that are divorced, and 47 (69.1%) that were widows. More infection was found among the divorced (71.1%) followed by the widow (69.1%), and married (45.9) with the divorced having the highest prevalence of HPV using the ELISA test. There was statistically significant difference in the result ($\chi^2 = 78.021$, $P < 0.05$).

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

Marital Status	Examined n (%)	HPV		χ^2	P-value
		Positive n (%)	Negative n (%)		
Married	231 (57.8)	106 (45.9)	125 (54.1)	78.021*	<0.001
Single	63 (15.8)	0 (0.0)	63 (100.0)		
Divorced	38 (9.5)	27 (71.1)	11 (28.9)		
Widow	68 (17.0)	47 (69.1)	21 (30.9)		
Total	**400 (100.0)**	**180 (45.0)**	**220 (55.0)**		

($\chi^2 = 78.021$, $P < 0.05$).

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 the ELISA test were also positive using PCR, 8 (29.6%) were divorced and

11 (23.4%) were widows. More infections were found among the divorced (29.6%) using PCR with specific primers to HPV type-16 infections. There is statistically significant difference in the result ($\chi^2 = 31.132$, $P < 0.05$).

Using HPV type-18 primers, some of the individuals that were tested positive using the ELISA test were also positive using PCR, 17(36.2%) and they are all divorced. There is statistically significant difference in the result ($\chi^2 = 53.124$, $P < 0.05$).

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

Marital Status	Examined n (%)	HPV-16			HPV-18		
		Positive n (%)	Negative n (%)	χ^2 (P-value)	Positive n (%)	Negative n (%)	χ^2 (P-value)
Married	106 (45.9)	0 (0.0)	106 (100)	31.132 (P < 0.05)	0 (0.0)	106 (100)	53.124* (P < 0.05)
Single	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Divorced	27 (71.1)	8 (29.6)	19 (70.4)		0 (0.0)	27 (100)	
Widows	47 (69.1)	11 (23.4)	36 (76.6)		17 (36.2)	30 (63.8)	
Total	**180 (45.0)**	**19 (10.6)**	**161 (89.4)**		**17 (9.4)**	**163 (90.6)**	

($\chi^2 = 31.132$, $P < 0.05$) ($\chi^2 = 53.124$, $P < 0.05$).

Table 9 shows the seroprevalence of HPV according to place of residence using the ELISA test. A total number of 400 individuals were examined using ELISA Test, 313 (78.3%) of the individuals were residing in an urban area and the other 87 (21.8%) of the individuals reside in a rural area. Out of the 180 (45.0%) positive individuals, 147 (47.0%) were residing in an urban area whereas 33 (37.9%) positive individuals reside in a rural area. There was more infection found among the urban dwellers (47.0%); however, it is not statistically significant ($\chi^2 = 2.245$, $P > 0.05$).

Table 9. Seroprevalence of human papillomavirus according to place of residence using ELISA test.

Place of Residence	Examined n (%)	HPV		χ^2	P-value
		Positive n (%)	Negative n (%)		
Urban	313 (78.2)	147 (47.0)	166 (53.0)	2.245	0.134
Rural	87 (21.8)	33 (37.9)	54 (62.1)		
Total	**400 (100.0)**	**180 (45.0)**	**220 (55.0)**		

($\chi^2 = 2.245$, $P > 0.05$)

Table 10 shows the seroprevalence of HPV according to place of residence using PCR with specific primers to the high-risk types. Out of the 180 (45%) positive individuals for the ELISA test, 147 (47%) were urban dwellers and 33 (37.9%) were rural dwellers. 13 (8.8%) of the urban dwellers tested positive to HPV using HPV type-16 specific primers and 6(18.2%) of the rural dwellers tested positive to HPV

type-16 using specific primers with the Urban dwellers having more infections but not statistically significant ($\chi^2 = 2.489$, **P= 0.115**).

Again, using PCR with specific primers to HPV type-18. Out of the 180 (45%) positive individuals to ELISA test kit, 147 (47%) were urban dwellers, and 33 (37.9%) were rural dwellers. 13 (8.8%) of the urban dwellers tested positive using HPV type-18 specific primers and 4 (12.1%) of the rural dwellers tested positive using the same primers with the Urban dwellers having more infection 13(8.8%) but not statistically significant ($\chi^2 = 0.339$, **P = 0.561**).

Table 10. Shows the prevalence of HPV according to place of residence using PCR.

Place of Residence	Examined n (%)	HPV-16			HPV-18		
		Positive n (%)	Negative n (%)	χ^2 (P-value)	Positive n (%)	Negative n (%)	χ^2 (P-value)
Urban	147 (47.0)	13 (8.8)	134 (91.2)	2.489 (0.115)	13 (8.8)	134 (91.2)	0.339 (0.561)
Rural	33 (37.9)	6 (18.2)	27 (81.8)		4 (12.1)	29 (87.9)	
Total	**180 (45.0)**	**19 (10.6)**	**161 (89.4)**		**17 (9.4)**	**163 (90.6)**	

($\chi^2 = 2.489$, P= 0.115) ($\chi^2 = 0.339$, P = 0.56).

Table 11 shows seroprevalence of HPV according to the number of sexual partners they had using ELISA test. Out of the 180 (45%) individuals that were tested positive to ELISA test, 363 (25%) claimed to have less than two partners, 37 and the rest did not state the numbers of sexual partners they had. 151 (41.6%) of the individuals with less than five sexual partners were positive using ELISA test kit and none of those that did not state the number of sexual partners they had tested positive. The result is significant statistically ($\chi^2 = 18.353$, **P < 0.05**).

Table 11. Seroprevalence of human papillomavirus according to number of sexual partners using ELISA test.

Sexual Partners	Examined n (%)	HPV		χ^2	P-value
		Positive n (%)	Negative n (%)		
≤2	363 (90.7)	151 (41.6)	212 (58.4)	18.353	<0.001
3 - 5	37 (9.3)	29 (78.4)	8 (21.6)		
Total	**400 (100)**	**180 (45)**	**220 (55)**		

($\chi^2 = 18.353$, P < 0.05).

Table 12. Prevalence of HPV according to the number of sexual partners they had using PCR.

Sexual Partners	Examined n (%)	HPV-16			HPV-18		
		Positive n (%)	Negative n (%)	$\chi2$ (P-value)	Positive n (%)	Negative n (%)	$\chi2$ (P-value)
≤2	151 (41.6)	3 (2.0)	148 (98.0)	72.888 (<0.001)	1 (0.7)	150 (99.3)	84.521 (<0.001)
3 - 5	29 (78.4)	16 (55.2)	13 (44.8)		16 (55.2)	13 (44.8)	
Total	**180 (45.0)**	**19 (10.6)**	**161 (89.4)**		**17 (9.4)**	**163 (90.6)**	

($\chi^2 = 72.888$, P < 0.05) ($\chi^2 = 84.521$, P < 0.05).

Table 12 shows seroprevalence of HPV according to the number of sexual partners they had using PCR with specific primers, out of the 180 (45.0%) individuals that tested positive using ELISA test, 3 (2.0%) of the individuals that had less than two sexual partners were positive to HPV type-16 specific primers and 16 (55.2%) of those individuals that have less than five partners were positive to HPV type-16 specific primers and more infection were found among them. There is statistically significant difference in the result ($\chi^2 = 72.888$, $P < 0.05$).

Using PCR with specific primers to HPV type-18. 1 (0.7%)) of the individuals that had less than two sexual partners were positive to HPV type-18 specific primers and 16 (55.2%) of those individuals with less than five partners were positive to HPV type-18 specific primers. More infections were found among those that have less than five partners 16 (55.2%). There is statistically significant difference in the result ($\chi^2 = 84.521$, $P < 0.05$).

4. Discussion

This study evaluated the seroprevalence of Human papillomavirus IgG antibodies from the samples collected from 200 people living with HIV and 200 HIV negative individuals. The samples that tested positive to Human papillomavirus antibodies in ELISA test were further analysed using Polymerase chain reaction to confirm the presence of Human papillomavirus type-16 and type-18. Most of the blood samples that tested positive to Human papillomavirus type-16 and type-18 infections were from confirmed HIV-positive individuals 159 (88.3).

Human papillomavirus was detected in 180 (45%) individuals using ELISA test, while 19 (10.6%) and 17 (9.4%) tested positive using PCR with the specific primers to HPV type 16 and HPV type 18 respectively in the studied individuals. ELISA IgG test detects the presence of specific antibody to human papillomavirus of different type both the high and low risk types. And using ELISA test which had a prevalence of 180 (45%) of positivity detected the presence of human papillomavirus immunoglobulin antibody (IgG) which only confirms the presence of human papillomavirus generally and not type specific. The test detected the presence of both the low and high-risk types meaning that it could be any of the more than 100 known types of human papillomavirus. Some of the human papillomavirus types only causes warts on the individuals and resolves itself on its own and these types are known as the low risk types because they do not progress to life threatening diseases like cervical cancer.

The results obtained with the ELISA test that detected the high prevalence rate 180 (45%) of the presence of specific IgG to Human papillomavirus established the association of Human papillomavirus as a common infection in HIV positive individuals in this part of the world which are mainly the low risk types. The result agrees with the study carried out by Ngwu and Ezeifeka in 2015 using ELISA only [19].

The seroprevalence of HPV according to age using ELISA test shows that individuals between the age group of 51 - 60 years 85 (47.2%) and 41 - 50 years 55

(30.6%) years have higher prevalence rate and this agrees with other studies. This also showed that there is a reduction in the percentage of the infection within this area compared to previous study that was done which could be as a result of sensitization awareness of the dangers of the infection if not properly managed.

Detection of HPV-16 and HPV-18 infections using human papillomavirus specific primers with higher prevalence as age increase agrees with the findings in prevalence data from Central and South America, Mozambique, Senegal, South Africa, in women in Ibadan Nigeria and another study from rural Nigeria where HPV was detected in high prevalence as the age increases [20].

On polymerase chain reaction (PCR) test, the result of individuals in the age range of 51 - 60 years were noted to be the most affected by the high-risk serotypes to cervical cancer HPV types-16 and type-18. This agrees with previous studies that noted higher prevalence of cervical cancer among women in advanced age [21] [22].

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 activation and mobilization of immune-active 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. This study agrees with a similar study done at Ibadan, Nigeria which also noted significant difference in prevalence of HPV among people living with HIV and HIV negative individuals [22].

In relation to the association of HPV seropositivity of the respondents to occupation, this study revealed high level of seropositivity by ELISA test among Hoteliers and traders. This may be attributed to engagement in lifestyles that increase their exposure to multiple sexual partners as corroborated by a similar study which noted that there was increased level of promiscuity.

Marital status is another socio -demographic factor that significantly affect 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 centre 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 [23] [24]. And HPV infection is being transmitted through the same route as HIV [25] [26].

Furthermore, respondents living in Urban areas were noted to have higher level of antibody sensitivity for HPV on ELISA. Most times, people in urban live a lifestyle that exposes them to risk especially risk to oncogenes. they are prone to engaging in risky behaviours that predispose them to diseases and other dangers due to high class life style.

Again, before attaining the high echelon of academic altitude, females who engaged in risky sexual behaviour early in life even at primary or secondary school levels can get the disease, hence the recent advocacy for early vaccination of female children from the age of 9 years - the catch them young approach.

All the positive results using both ELISA and PCR were mostly HIV positive females, making the assumption that due to the impaired immune status of HIV-positive women, they are likely to have higher rates human papillomavirus of infections than seen in the general population of this region. The high-risk HPV detected in our study among HIV positive women is also similar to the types found in other West African countries and other African countries like Zambia [27] [28] And agrees with the worldwide prevalence rate of HPV-16 and-18 [29].

From the results, it was obsersved that the number of samples where HPV antibodies were detected was much more than the positive results from PCR. This is significant, however, because ELISA detected all antibodies to HPV types (both high risk and low risk types) whereas PCR done was specific for the high-risk HPV that is type-16 and type-18. This finding has important role in the eventual implementation of prophylactic human papillomavirus vaccines based on high risk type-16 and type-18.

This work agrees that HIV-infected individuals are also at risk of other deadly infections although HPV infections are often mild except the one of high-risk types.

Infection with Human papilllomavirus is life-long and many infected individuals are unaware of this infection and constitute high risk in transmitting and spreading the disease. Specific preventive strategies such as: avoiding multiple sexual partners should be encouraged since it is a major source of transmission. The virus is shed in body fluids hence assessing serologic status between sexual partners may assist in Human papilllomavirus prevention.

The study showed that there is higher human papillomavirus among people with lower economic background.

This study highlights the importance of cervical screening programmes among

women irrespective of their HIV status as this helps in reducing the burden of HIV and HPV infection and consequently contributes to curbing of the menace of cervical cancer in our society.

Vaccines currently have been developed for the control of HPV but due to its cost many individuals shy away from it. The best option to reduce the circulation of HPV in the population would be to make the vaccine available at an affordable cost or if possible free and immunize every girl child from the age of nine and above.

Measures to improve awareness of HPV screening are by integration of HPV Vaccination into routine immunization program, enhancing improvement on the awareness campaign through health education program in schools, and adopting implementation of government free HPV Screening program especially in the rural areas.

5. Conclusions

There is evidence of significant exposure to HPV type-16 and HPV type-18 in the study population. Therefore, it remains important to closely monitor HPV-related disease in women with HIV, particularly in this region of the world where cervical screening is not routinely available. Antiviral medications given for episodic outbreaks or as long-term suppressive treatment provide important clinical benefits to patients. Given the apparent epidemiologic synergy between HPV and HIV, promoting awareness of HPV screening for the purpose of decreasing HPV transmission and disease progression has substantial public health benefits. Human papillomavirus screening has reduced both the number and deaths from cervical cancer in the developed world and this study exposes the need for human papillomavirus screening because it will help in detecting early infections that may develop into cancer. This allows for early management which results in better outcomes.

The result of this study will help physicians taking care of HIV patients and other non-positive individuals to know how common or otherwise Human papillomavirus infection is among HIV patients and negative individuals, hence stand in a better position to make more accurate diagnoses in order to reduce cancer morbidity and mortality since cancer screening is not among the routine test or common test done. This study will make them know that there is a need for them to inculcate human papillomavirus screening as one of the vital tests.

In view of the high prevalence and diversity of HPV genotypes among HIV positive women, adequate screening protocols should be put in place for screening this category of women. Studies should also be carried out to determine the efficacy of existing HPV vaccines on this group of patients.

As this study was hospital based, a population-based study should be carried out to reveal some vital information which could provide an important insight into the hidden dynamics of this infection. It is recommended that all HIV-positive and non-positive individuals should be screened for HPV especially of the

high-risk types (HPVtype-16 and type-18) infections. And if the results turn out to be HPV-positive, the individuals should be placed on HPV topical drugs and should be managed properly in order to reduce the risk of progressing to cervical cancer.

Declarations

Ethical Approval and Informed Consent

Ethical clearance with reference number (**AE-FUTHA/REC/VOL 4/2023/427)** was obtained from Alex-Ekwueme Federal University Teaching Hospital Abakaliki. All participants were duly informed of the objectives of the study and the protocol for sample collection. Participation was voluntary.

Authors Contribution

All the authors contributed to this research work starting from the beginning of the research to the stage of developing the manuscripts and approved its submission.

Conflicts of Interest

Authors declared that there was no conflict of interest.

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