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Research Article

Prevalence and Possible Risk Factors for Human Papilloma Virus Among HIV Infected Women

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Abstract

Human papilloma viruses (HPV), are DNA viruses that infect the cutaneous and mucosal epithelia, manifesting as non-genital or genital warts, and pre invasive and invasive lesions. HPVs infecting the anogenital tract are divided into high risk and low risk types, based on their association with malignant or benign disease. A cross sectional study involving 50 HIV-I infected females randomly recruited from the HIV clinic of St Charles Borromeo Hospital Onitsha was conducted. Ethical approval was obtained then blood and cervical swab samples were collected. The following were determined; HIV screening and confirmation, CD4 count, and HPV. Data analysis was carried out using the SPSS version 20. Out of the 50 women who were screened for the presence of HPV genotypes, 3(6%) were positive for various HPV genotypes and were aged 25-44 years. Only the participant's CD4 count was found to be significant for acquisition of HPV infection with $P<0.05$. This relates only to those with CD4 < 200cells/mm^3 and 200-350 cells/mm^3. The participant's age range and WHO stage of disease were not significant risk factors for acquisition of HPV infection with $P>0.05$. Finally, high risk HPVs were more prevalent in the population studied, therefore it may be wise to administer HPV vaccines to younger uninfected females since it is protective for the dominant high risk groups. It is also recommended that this research be carried out on a larger scale so that more HPV genotypes circulating in the society can be discovered to aid the production of update vaccines.

Keywords: Prevalence, HPV, HIV, Genotype , Risk factors

INTRODUCTION

Over 150 different types of Human Papillomavirus (HPV) have been identified, of which about 40 are known to infect the anogenital tract (Munoz et al, 2006). HPV's are DNA viruses that infect cutaneous and mucosal epithelia, manifesting as non-genital or genital warts, and preinvasive and invasive lesions. HPVs infecting the anogenital tract are divided into high-risk and low-risk types, based on their association with malignant or benign disease. HPV infection is associated with almost all cervical cancers, but also with other cancers of the anogenital tract including cancer of the vulva, vagina, anus, penis and some head and neck cancers. Human papillomavirus particles consist of 8000 base-pair (bp) long circular DNA molecules wrapped into a protein shell composed of two molecules (L1 and L2). The viral genome has the coding for these two proteins and the six early proteins (E1, E2, E4 –E7), that are necessary for the replication of the viral DNA and for the assembly of the newly produced viral particles within infected cells. Both sets of genes are separated by an upstream regulatory region (URR) of about 1000 bp that does not code for proteins but regulates gene expression, replication of the genome, and its packaging into viral particles (Munoz *et al*., 2003). HPV particles reach the basal layer of the epithelium, where they bind to and enter cells through microtrauma. Within the epithelium, the viral genome is replicated to a copy number of about 100 and maintained for varying periods of time at this low copy number. The viral proteins E1 and E2 are essential for this basal DNA replication. Once the basal cells are pushed to the suprabasal compartment, they lose their ability to divide and initiate the terminal differentiation program. The critical molecules in the process of virus replication are the viral proteins E6 and E7, which interact with a variety of cellular proteins (Munoz *et al*., 2003). The best characterized interactions are with proteins pRB and p53. Binding of E7 to pRB activates the E2F transcription factor, which triggers the expression of proteins necessary for DNA replication. The E6 protein targets p53 for proteolytic degradation. As a consequence the dependence on cell cycle control is abolished and normal keratinocyte

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differentiation is retarded. As a result of virus infection, constant activity of the viral proteins E6 and E7 leads to increasing genomic instability, accumulation of oncogene mutations, further loss of cell-growth control and ultimately malignant transformation.

The global public health burden attributable to Human Papilloma viruses is considerable.HPV poses an important public health threat amongst HIV positive women. The most important known determinant of HPV resistance and progression to cancer is most HPV type 16 and 18 which are strongly associated with high grade squamous intraepithelial lesion and 52 which is the seventh most frequently detected as high risk type in cervical cancer worldwide (Clifford et al ,2006). HIV infected women are more likely to be infected with HPV than HIV negative women. Data from the American Women's Interagency HIV study (WIHS) found that 58% of HIV infected women had HPV compared with 24% of HIV negative women (Palefsky, 1999). The WIHS reported that advanced HIV disease was strongly associated with HPV infection. In general, the prevalence of HPV increases with progressive reduction of CD4 cells (Palefsky, 2007) and the presence of multiple types can also increase with the progressive CD4 (Palefsky, 2007) reduction. Little is known about the natural history of HPV infection in HIV positive women and persistent of HPV infections may explain the increased risk of cervical squamous intraepithelial lesions and invasive cervical cancer in HIV positive women (Strickler,2005). Notably, the presence of HPV immune suppression by HIV infection also appears to worsen the outcome of HPV infection. Women infected with HIV are at significantly increased risk of invasive cervical cancer which cannot be explained purely by higher incidence of HPV infection among these women.

The two currently available vaccines that protect against the acquisition of hr HPV are based on HPV 16 and 18, the reported most common HPV genotypes globally (Okonofua, 2007). However, there is increasing evidence of regional and sub-regional variations in HPV genotype distribution, suggesting that the current vaccines may not be as effective in the sub-Saharan Africa region as projected (Blossom et al, 2007) .Previous studies from sub-Saharan Africa show that although the burden of HPV is high compared to Europe and North America, a lower prevalence of HPV 16 and 18 and a higher prevalence of other hr HPV genotypes such as HPV 31, 35 and 58, were observed (Okonofua, 2007). A plausible assumption could therefore be that other non-HPV 16 and 18 oncogenic genotypes may account for thousands of new cases of cervical cancer that occur in this region annually. In addition, since immunity to HPV virus like particle vaccines is type-specific, it is critical to characterize the distribution of hr HPV genotypes in the West African sub region with high burden of both HIV and HPV infection, in order to develop an effective vaccine targeting women in this region (Okonofua, 2007). Unfortunately, several low-income countries, including Nigeria, have approved and deployed the HPV vaccine without such a detailed characterization of the HPV genotypes in their settings (NACA, 2011). Considering the amount of resources invested in procuring and distributing this vaccine, it is imperative to conduct studies that will determine the prevalence of prevailing HPV genotypes in these countries. Such information will potentially contribute to the development of second-generation HPV vaccines, which will improve prevention and control of cervical cancer in the sub-region (Blossom et al, 2007). This study will therefore identify some of the HPV genotypes plaguing women infected with HIV in our society so that adequate awareness can be created for vaccine intervention.

MATERIALS AND METHODS

Study Design: A cross sectional study was conducted among 50 HIV-I infected females randomly recruited from the HIV Clinic of St Charles Borromeo Hospital Onitsha. Ethical consideration was obtained from the Ethical committee of the hospital. Informed consent was obtained from the patients after which they were served questionnaires. Questionnaires were accurately filled and the following data obtained; personal factors like age, gender, WHO criteria, Marital status, State and Local government of origin. Five milliliters of blood samples were collected using EDTA anticoagulant bottles and plasma was later obtained after centrifugation. Cervical swabs were also collected for HPV genotyping. The results obtained were analysed using SPSS version 20.

Inclusion Criteria: Participants were selected if they tested positive to HIV-I **.** If they are females and also within the age range of 18-64 years

Exclusion Criteria: Participants were excluded if they test negative to HIV-I, If their ages are not within the age range of 18-64 years or if they are pregnant.

Study Area: The study area was Onitsha in Anambra state Nigeria. Onitsha is located beside the popular river Niger. People here mostly engage in business and few are civil servants.

Calculation of Sample Size: Sample size was calculated using the minimum sample size for simple proportion with 5% margin of error and 95% level of confidence according to the method of Daniel , 1999.

$$N=Z^2PQ/D^2$$

Where Z= Standard normal deviation at 1.96(which corresponds to 95% confidence interval).

P= Prevalence of HPV infection in HIV Patients =4.2% (Oboma and Avwioro, 2012)

$$Q=1-P$$

D=Degree of accuracy/ precision expected = 0.05%

Substituting for the above formulae

$$N=1.96^2x(0.042x0.958)/(0.05)^2 = 61$$

Hiv Rapid Testing: The rapid HIV test kits consisting of 3 different antigens were used for the diagnosis of HIV infection by serial testing algorithm. The first test was performed using Determine kit (Abbott Laboratories, USA) (screening test) and if positive a second test was performed using Unigold(Trinity Biotech, Ireland), for positive samples, the patient is considered positive for HIV but if negative, the third test which acts as tie breaker is carried out using Stat pak (Chembio Diagnostic System, USA) as recommended by the National HIV rapid test Algorithm.

CD4 Count: The CD4+ T cell count was done to determine the level of immune function. The Cyflow Partec machine (Partec GmbH, Munster, Germany) were used. The Cyflow

detects and identifies fluorochrome- labeled (phytoerythrin, PE) cells as they pass through an optical detecting system by detecting the angle of scatter of incident laser and the wavelength of fluorescence emitted.
Twenty microlitres of patient's blood was added to 20µl of PE antibody in Rohren tube. This was adequately mixed and incubated in the dark for 15mins at room temperature. Exactly, 800µl of the buffer was then added to the tube and mixed adequately. The sample tube was plugged onto the sample port of the Cyflow machine for counting of the CD4 + T cells. The monitor displayed the result of the counting and this was recorded as the number of cells/µl of blood.

Purification Of Cervical Swab Specimens: Cervical swab samples were asceptically collected by a gynecologist. The dry swabs from the patients were directly put into a 1.5 ml microcentrifuge tube and vortexted with 1,000 µl 1 x PBS for 15 seconds. Finally, 200 µl of the suspended cervical swab was put into 1.5 ml microcentrifuge tube and this was used for genomic DNA extraction. The cervical swab samples were transported (Using PBS as transport medium) in a cold chain maintained at -6^{o}C using ice packs to the Immunology Laboratory, Department of Microbiology Nigerian Institute for Medical Research where they were stored at -20^{o}c upon reception and then assayed .

Human Papilloma Virus DNA Extraction: This was performed using the ZR Genomic DNA Tissue Miniprep (Zymo research). The ZR Genomic DNA Tissue Miniprep is a simple procedure for the rapid isolation of total DNA (e.g genomic, mitochondrial, parasitic, microbial, viral) from a variety of solid tissues. The genomic DNA was extracted according to Manufacturer's instructions.

DNA Amplification: This was performed using the Seegene HP1400Y Seeplex HPV4 ACE kit. The Seeplex HPV4 ACE Screening kit is a qualitative in vitro test for the detection and screening of HRC [High Risk Common] and HPV-16, HPV-6/11, HPV-18 .It is a multiplex kit. The 2X Multiplex Master Mix contains dNTP and enzyme for the specific amplification of the virus genome and 5X HPV4 ACE PM is primer mixture for initiation of PCR. In PCR, efficiency can be reduced by inhibitors that may be present in the clinical specimen. The Internal Control has been added to the Seeplex HPV4 ACE Screening to identify processed specimens containing substances that may interfere with PCR amplification. The Internal Control is a DNA plasmid. These features were selected to ensure equivalent amplification of the Internal Control and HPV DNA. The Internal Control is introduced into each amplification reaction and is co-amplified with target DNA from the clinical specimen. In addition, the 8-methoxypsoralen (8-MOP) system is used to extinguish the template activity of contaminated DNAs. 8-MOP is known to intercalate into double-stranded nucleic acids and form a covalent interstrand crosslink after photo activation with incident light of wavelength of 320-400 nm.

Protocol For Preparation Of PCR Mastermix
The tubes were then vortexed , and centrifuged briefly. Then 17 µl of the PCR Mastermix (consisting of 4 µl 5X HPV4 ACE PM, 3 µl 8-Mop Solution and 10 µl 2X Multiplex Master Mix)was put in 0.2 ml PCR tubes and the tubes closed. This is followed by addition of 3 µl of each sample's nucleic acid.The final volume is now 20 µl. A new pipette tip was used for each sample. For the negative control PCR, 3 µl of the distilled water was used instead of sample's nucleic acid. Then for the positive control PCR, 3 µl of the HPV4 ACE PC was used instead of sample's nucleic acid

Programme for PCR Amplification: The tubes were placed in a preheated (94℃) thermal cycler. It is important to preheat (94℃) the thermal cycler before placing the tube in the thermal cycler for hot starting. Cycling were as follows; first segment single cycle at 94^{o}C for 15min, followed by 40 cycles at 94^{o}C for 0.5min, 60^{o}C for 1.5min 72^{o}C for 1.5min and a final segment of one cycle at 72oC for 10min in Thermo cycler (Germany)

Agarose Gel Electrophoresis/ Band Visualisation: Electrophoresis of 5 ul of the PCR PRODUCT and 5ul of HPV4A ACE Marker on 2% agarose gel containing 10ug/ml of ethidium bromide was done at 70V. The gel was viewed using a gel documentation system.

RESULTS

Plate 1 shows the bands of amplification of purified DNA in a gel. The positive and negative controls have been included. The positive control which is the DNA marker have several bands for reference purpose.. The first on the well is the internal control. This is followed by various bands being markers for HPV 16, HRC, HPV 6/11, and HPV 18 which is most distant from the well. Two of the study participants had their DNA samples amplified for HPV as shown in this gel. While sample 247 is positive for HRC, 249 shows that the participant is positive for HPV 16.

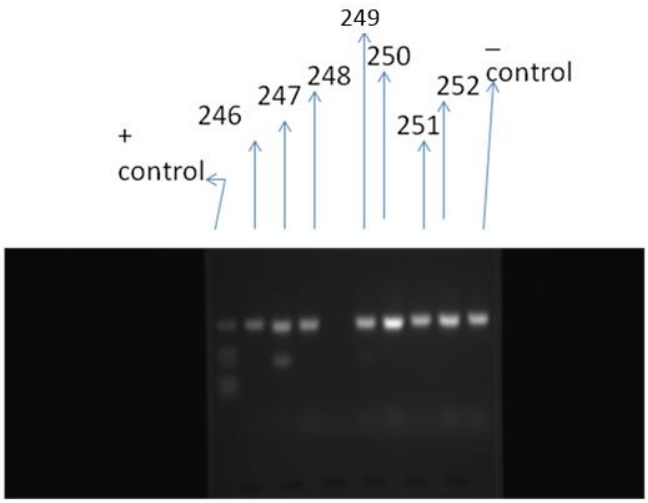

Plate 1:
Bands of the amplified HPV DNA. While band 247 is positive for HRC, band 249 is positive for HPV 16.

Results from Plate 2 show a sample with multiple genotypes. The multiple bands refers to HPV 16, HPV 18 and HPV 6/11. The positive , negative and internal control bands are shown.
As shown in Figure 1, Here the prevalence of Human papilloma virus among the various age groups of HIV positive women was shown. Three (6 %) tested positive for various HPV genotypes and were aged 25-44 years. Among those

tested, 47 women tested negative and includes 42(93.3%) in the 25-44 years age group and 5(6.7%) in the 45-64 years age bracket.

The possible risk factors for HPV is shown in Table 1. Various factors including CD4 count, Marital status, age range and WHO stage of patients HIV disease were assessed as possible risk factors for acquisition of HPV infection . Only the patients' CD4 count was found to be significant for acquisition of HPV infection with P<0.05.

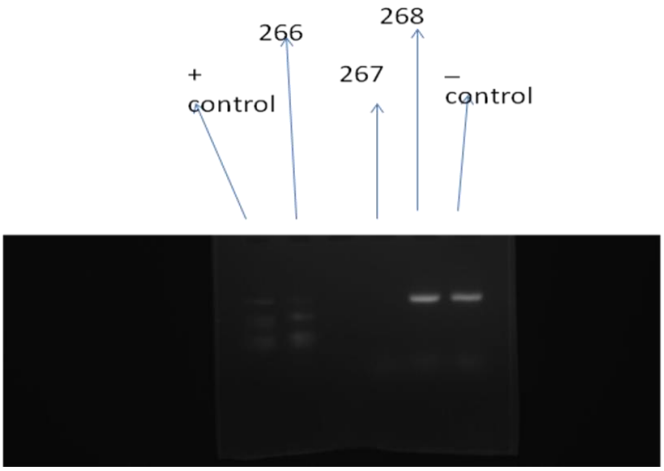

Plate 2:
Bands of the amplified HPV DNA. Sample 266 shows infection with multiple genotypes of HPV namely 16, 18 and 6/11 hence the multiple bands

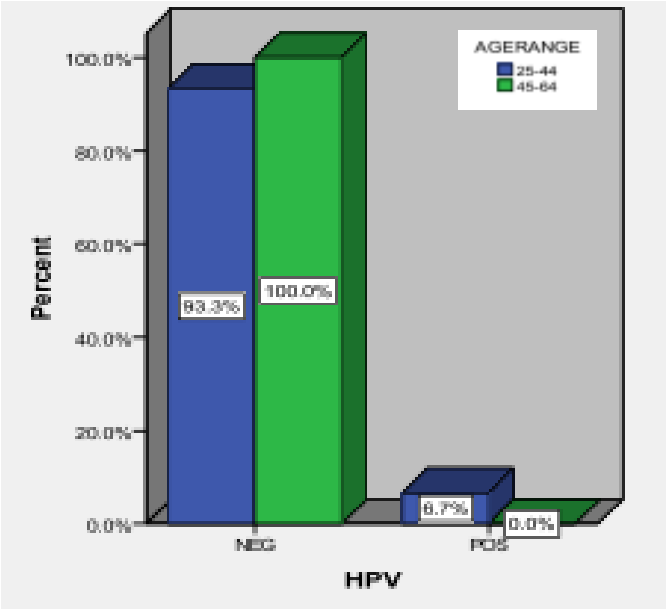

Fig 1:
Prevalence of Human papilloma virus in various age groups of HIV positive women

Table 1:
Possible Risk Factors For HIV/HPV Co-Infection

Possible Risk Factors		HIV Positive Only No (%)	HIV/HPV Co-Infection No (%)	P-Value
CD4 RANGE(cells/mm^3)	<200	2(4.3)	2(66.7)	0.001
	200-350	18(38.3)	1(33.3)	
	351-500	9(19.1)	0(0)	
	>500	18(38.3)	0(0)	
MARITAL STATUS	MARRIED	19(40.4)	1(33.3)	0.538
	SINGLE	18(38.3)	2(66.7)	
	WIDOWED	10(21.3)	0(0)	
AGE RANGE(yrs)	25-44	42(89.4)	3(100)	0.724
	45-64	5(10.6)	0(0)	
WHO	STAGE 1	36(76.6)	1(33.3)	0.128
	STAGE 2	2(4.3)	1(33.3)	
	STAGE 3	9(19.1)	1(33.3)	

Results are presented as Means ± S.D. P value is significant at P<0.05
Only CD4 count was significant among the possible risk factors

DISCUSSION

Out of the 50 women screened for the presence of HPV genotypes, 3(6 %) tested positive for various HPV genotypes .They are aged 25-44 years . This result is higher than the 4.2% obtained by Oboma and Avwioro, (2012), in Bayelsa but lower than the 19.6% reported by Ezechi et al, (2014), in Lagos. Among those tested, 47 women tested negative and includes 42(93.3%) in the 25-44 years age group and 5(6.7%) in the 45-64 years age bracket . Sexual contact at young age increases the likely hood of HPV infection and increases the time for (HPV) infection to progress to pre-cancerous changes and eventually to invasive cancer especially in HIV positive (Mike and Henry, 2001). HPV infections are more likely to persist in HIV positive women than in HIV negative women and this persistence contributes to a higher prevalence of HPV infection among HIV positive women and a higher risk of developing squamous intraepithelial lesions (Vem *et al*., 2012) . In Jos Nigeria, Vem et al, (2012), looked at the distribution of premalignant changes in the HIV positive women. Their results showed that about 9.0% were infected with HPV while only 6.0% were infected with HPV among HIV negative women. This suggests that, infection with HIV is an important risk factor for HPV infection and development of HPV associated lesion in the female genital tract and also immune

damage caused by HIV increases the risk of developing cervical cancers. Some possible risk factors associated with HIV/HPV co-infection were examined . Various factors including CD4 count, Marital status, age range and WHO stage of patients HIV disease were assessed as possible risk factors for acquisition of HPV infection . Only the patients' CD4 count was found to be significant for acquisition of HPV infection with $P<0.05$. This relates only to those with CD4 <200cells/mm^3 and 200-350 cells/mm^3 (i.e low CD4 ranges). The patients marital status, age range, and WHO stage of disease were not found to be significant risk factors for acquisition of HPV infection with $P>0.05$.This shows that HPV dominated among participants with low CD4 counts. HIV-positive women and men have more genital HPV compared to HIV-negative women and men, respectively, and low CD4 count influence HPV prevalence (Riva *et al*, 2007). HPV incidence is reported to be high in HIV-positive individuals due to a suppressed immune system (Strickler *et al*., 2005). Generally, LR HPV types are more prevalent among HIV-negative men compared with HIV-negative women (Srickler *et al*., 2005). In this study, it was found that high risk HPV were prevalent in HIV infected women, therefore it may be wise to offer HPV vaccines to younger uninfected females since it is protective for the dominant high risk groups. It is recommended that this work be carried out on a larger scale so that more HPV genotypes plaguing women can be discovered for an effective production of update HPV vaccines

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