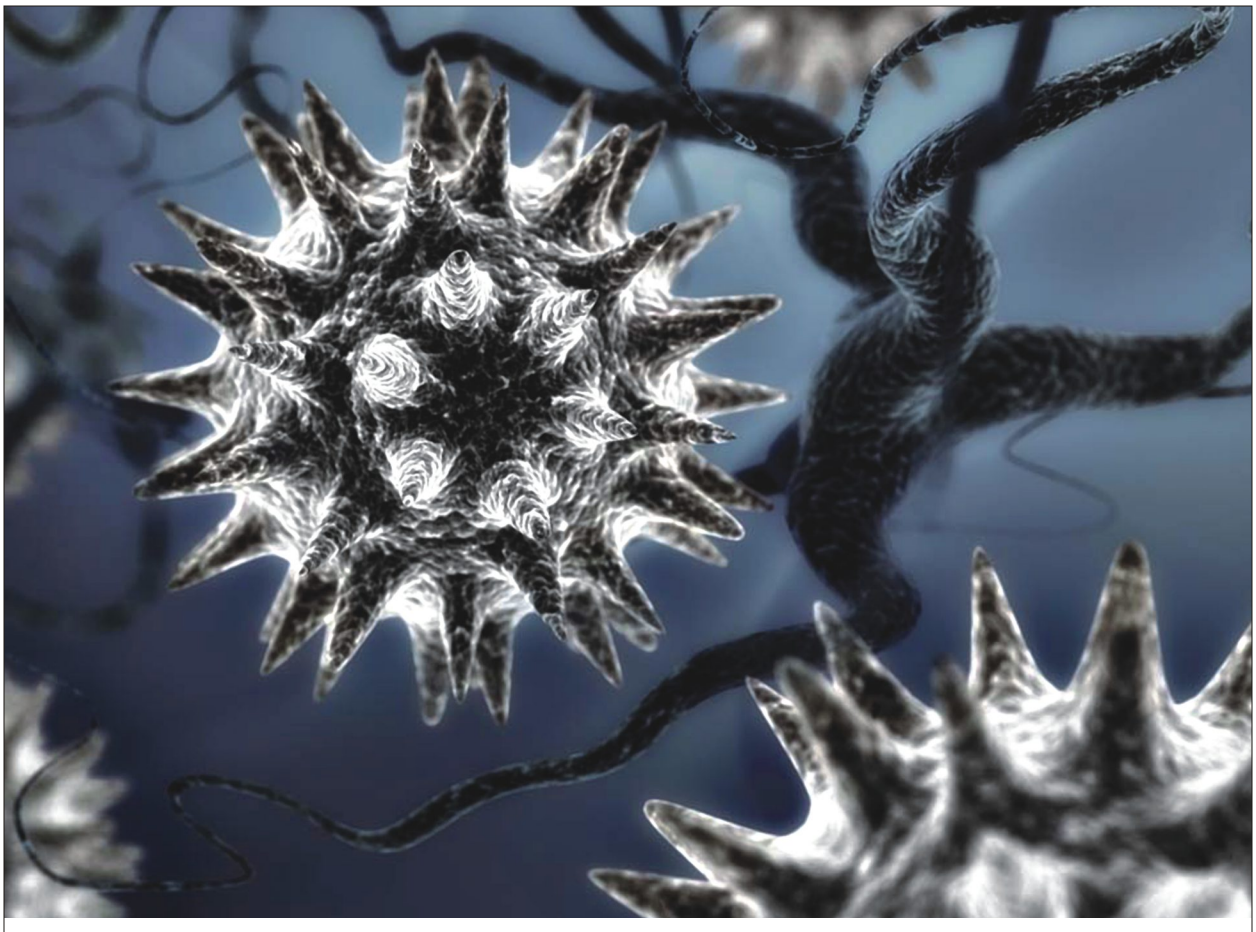

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Research Article
Seroprevalence and Molecular Investigation of Human Papillomavirus (HPV) Type-16 in HIV Positive Women in Abakaliki, Nigeria

[1]Euslar Nnenna Onu, [2]Emmanuel Mbah Onu, [3]Chika Ejikeugwu, [4]Cornelius Ituma Azi, [5]Jude Ugwu and [6]Ogbonnaya Ogbu

[1]Department of Medical Microbiology, Alex Ekwueme Federal University Teaching Hospital Abakaliki, Nigeria
[2]Department of Chemical Pathology, Alex Ekwueme Federal University Teaching Hospital Abakaliki, Nigeria
[3]Department of Pharmaceutical Microbiology and Biotechnology, Enugu State University of Science and Technology, Agbani, Nigeria
[4]Department of Emergency, Al Shamli General Hospital, Hail, Saudi Arabia
[5]Department of Medical Laboratory, Alex Ekwueme Federal University Teaching Hospital Abakaliki, Nigeria
[6]College of Basic Medicine, Ebonyi State University, Abakaliki, Nigeria

Abstract

Background and Objective: Early and routine detection of HPV which is responsible for most cervical cancers in women infected with HIV could not only improve their prognosis but can help in reducing the mortality and morbidity associated with such populations. The HPV infections are caused by a variety of HPV types including HPV-16. This study investigated the prevalence of HPV-16 in HIV-infected women in Abakaliki, Nigeria. **Materials and Methods:** Whole blood samples (aliquots of 10 mL) were aseptically collected from HIV-positive women (n = 100) who attended a tertiary hospital in Abakaliki, Nigeria for antiretroviral therapy and counselling. Also, 60 HIV-negative women were included as a control group. All samples were investigated by ELISA and PCR for the detection of HPV-16. **Results:** A high prevalence of HPV antibodies (HPV IgG) in the HIV-1 positive women was reported. It was observed that there was a significant association of HPV-16 infection with age and sexual activity among the HIV-1 infected women. The prevalence of high risk HPV-16 (8.8%) in the patient's blood samples was reported in HIV-positive women. **Conclusion:** Given the prevalence of HPV-16 and HPV antibodies reported in this study, it could be concluded that HIV-1 infection could increase the susceptibility of women to HPV-16 infection, particularly among those already living with the immunodeficiency disease (HIV-1).

Key words: Human papillomavirus, sexually transmitted diseases, STDs, HIV-1, Nigeria

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Corresponding Author: Chika Ejikeugwu, Department of Pharmaceutical Microbiology and Biotechnology, Enugu State University of Science and Technology, Agbani, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Human papillomavirus 16 (HPV-16) that is categorized as a high-risk HPV associated with cervical cancer, belongs to the *Papillomaviridae* viral family[1,2]. The papillomaviruses including HPV-16 are known to be cancer-causing viruses that are implicated in human infections, both in females and males[2]. The HPV is a notable cause of cancer in women globally and infection with this virus is a common sexually transmitted disease common among female adolescents and young adults around the world. Nonetheless, the prevalence and transmission of this cancer-causing virus in human population in the developing countries like Nigeria is scarcely investigated. It is known that the basal keratinocytes of mucosal and cutaneous epithelia of both animals including birds, marsupials, reptiles and humans are infected by papillomaviruses[3]. According to the US Centre for Disease Control and Prevention (CDC), it is advisable to create awareness of HPV and ensure timely immunization of females, especially sexually active adolescence girls and young women in order to reduce the public health risks of HPV infection and cancer development in the human population[4]. The HPV infections are usually subclinical in nature and those infected show little or no clinical symptoms. Benign papilloma or malignant lesions that further complicates the disease process can also ensue from previously asymptomatic HPV infection[5,6]. The papillomaviruses are of immense clinical importance because of their ability to initiate the development of cancer in infected individuals[6], particularly in women and in immune-compromised individuals such as those living with HIV-1. There has been a reported incidence of HPV-16 infection as high as 60% across a range of populations, genders and habits. Genes E6 and E7 of the HPV-16 encode the two major oncoproteins E6 and E7, which are required for cell cycle transformation and virus immune system evasion of the infecting organism[7]. The HPV-associated cancers are usually common amongst patients[8,9]. It is therefore important to screen for HPV strains as a routine to guide therapy in such individuals in order to control the evolution and transmission of the virus in human population. Since sexually active females are prone to cervical infection with HPVs that may culminate into cancer, it is therefore critical to elucidate the prevalence of HPV-16 amongst people living with HIV/AIDS in local communities as panacea to make informed therapeutic measures for such target groups. Despite the fact that HIV-1 infection and cervical cancer co-infections are significant public health concerns, data on the seroprevalence and molecular detection of HPV-16 in HIV-positive women are scarce in Nigeria[10]. Hence, this study aimed to elucidate the seroprevalence of HPV-16 and its molecular detection among HIV-positive and HIV-negative females in Abakaliki, Nigeria.

MATERIALS AND METHODS

Ethics approval: This study received approval and ethical permission from the Research Committee of the Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria and the Research and Ethics Committee of the Federal Teaching Hospital Abakaliki, Nigeria [FTH-16/01/2016] in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants in this study.

Study area: Participants in this study were recruited from tertiary hospitals in Abakaliki Metropolis, Ebonyi State, South-Eastern Nigeria. Abakaliki is an agrarian metropolitan society located between longitude 7.30° and 8.30° East and latitudes 5.40° and 6.45° North[11].

Participants and samples: This study evaluated a total of 160 blood samples from both HIV-positive women (n = 100) as the test group and HIV-negative women (n = 60) as a control group in the age range of 18-65 years who referred to tertiary hospitals in Abakaliki Metropolis, Nigeria during January, 2019 to July, 2020. The status of HIV positivity was confirmed using previous existing records of patients. HIV tests including HIV Enzyme-Linked Immunosorbent Assay (ELISA), CD4 T lymphocytes levels, CD4/CD8 T lymphocytes ratio and HIV-RNA load were also performed. Blood samples (aliquots of 10 mL) were aseptically collected at the phlebotomy unit of the hospitals and these were each subjected to ELISA test and DNA extraction for Polymerase Chain Reaction (PCR) detection of HPV-16 according to the manufacturer's instructions.

Inclusion and exclusion criteria: The inclusion criteria for the study included women who were consenting HIV-positive and HIV-negative females between the ages of 18-65 years while the exclusion criteria included those who were males, children and females who were menstruating, pregnant, vaccinated against HPV and those who declined HIV testing.

HPV-16 detection by ELISA: The microtiter plate provided in the ELISA kit (Cusabio Co Ltd., Germany) was pre-coated with an antigen specific to detect the HPV-16 IgG antibody in the participants' sera samples. In accordance with manufacturer's

specifications, the test procedure was conducted and the results were interpreted based on previous protocol[10,12]. The intensity of colour change was measured spectrophotometrically at 450 nm using an ELISA reader (Tecan Group Ltd., Mannedorf, Switzerland). A cut-off value was determined based on the instructions provided by the manufacturer. The HPV-16 positive samples were defined as those whose optical density was 2.1 or greater in comparison with those of the negative controls. Negative samples were defined as those whose optical density fell below the cut-off ratio[13].

Detection of HPV-16 by PCR technique: The prevalence of HPV-16 in the samples was investigated by PCR using forward primer 5'-TTTTGGGTTACACATTTACAAG-3' and the reverse primer 5'-TGTCTGCTTTTATACTAACCG-3' according to the previous methodology[7]. DNA extraction was completed using the Viral Zymogen Kit (Zymo Research Corporation, USA) according to manufacturer's instructions. PCR was used to amplify the DNA of the HPV-16 present in the blood samples recovered from the HIV-positive and HIV-negative subjects investigated. The final volume of PCR mix (25 µL) comprised 3 µL of extracted DNA, 12.5 µL of PCR Master Mix (Ampliqon, Denmark), 0.5 µL (10 pmol) of each primer and 8.5 µL of distilled water. Initial denaturation of the PCR was performed at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min and a final extension step for 10 min at 72°C[7]. The products of the PCR gene amplification were run on 1.5 % agarose gel. The PCR products were visualized in a UV transilluminator.

Data analysis: Data were analysed 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 the two groups (HIV-positive women and HIV-negative women) in this study. The $p \leq 0.05$ were considered significant.

RESULTS

The HPV-16 IgG was identified by ELISA in 103 (64.4%) blood samples including 88 (85.4%) HIV-1 positive women and 15 (14.6%) HIV-negative women. The prevalence of HPV-16 IgG among the women investigated in this study based on their age was shown in Table 1. Significant higher levels ($p = 0.0001$) of HPV-16 IgG were recorded among HIV-positive women that were older in age (>40 years) than those of younger women (Table 1).

The positivity rate of HPV amongst the HIV-1 positive women based on their sexual activity or sexual partners was shown in Table 2. Women with less than 5 sexual partners showed lesser frequency of HPV type 16 compared to those who did not state their sexual partners (Table 2).

Nonetheless, those who did not disclose their sexual activity in terms of number of sexual partners had a higher prevalence of HPV type 16 (Table 2). The HPV type 16 was detected in 14 (8.8%) HIV-1 positive women by PCR compared to 103 (64.4%) HIV-1 positive women who were confirmed by ELISA to possess antibodies for HPV (Table 2), however, PCR is usually used as a confirmatory test for HPV rather than ELISA-which was usually used to determine the seroprevalence of HPV antibodies in infected individuals.

Table 1: Prevalence of HPV-16 by ELISA and PCR in various age groups

	HIV-positive women (n = 100) n (%)			HIV-negative women (n = 60) n (%)		
Age range (year)	HPV-16 ELISA positive	HPV-16 PCR positive	HPV-16 ELISA/PCR positive	HPV-16 ELISA positive	HPV-16 PCR positive	HPV-16 ELISA/PCR positive
≤ 20	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.0)	0 (0.0)	0 (0.0)
21-30	5 (5.0)	0 (0.0)	0 (0.0)	19 (31.7)	5 (8.3)	1 (1.7)
31-40	14 (14.0)	2 (2.0)	2 (2.0)	9 (15.0)	10 (16.7)	9 (15.0)
41-50	22 (22.0)	9 (9.0)	7 (7.0)	4 (6.7)	7 (11.7)	1 (1.7)
51-60	21 (21.0)	3 (3.0)	2 (2.0)	12 (20.0)	8 (13.3)	8 (13.3)
Total	62 (62.0)	14 (14.0)	11 (11.0)	47 (78.3)	30 (50.0)	21 (35.0)

Table 2: HPV positivity amongst HIV-1 positive women based on sexual activity

Number of sexual partner	Number examined n (%)	HPV type 16 positive n (%)	HPV type 16 negative n (%)
<5	11 (10.7)	3 (27.3)	8 (72.7)
5-10	0.00	0.00	0.00
>10	0.00	0.00	0.00
Not stated	92 (89.3)	11 (12)	81 (88)
Total	103 (64.4)	14 (13.6)	89 (86.4)

In addition, the positivity rate of HPV type 16 amongst women living with HIV/AIDS as confirmed by both ELISA and PCR in thus current study showcases the possible co-morbidity and poor prognosis of such individuals to treatment. This could further complicate the already immunocompromised state of the individuals and further speed up the process of establishing an invasive and severe type of cancer-since HPV could increase in populations of women already living with HIV/AIDS.

DISCUSSION

Women living with HIV/AIDS are at increased risk of developing cervical cancer due to the risk of HPV co-infection since such population of women is already experiencing an immunocompromised state of health which could further plunge them into a debilitated state of health. Several studies have shown that the population of women already living with HIV/AIDS are prone to co-infections from other pathogens and simultaneous infections from the HPV types, particularly HPV16 or HPV type 16-which is a notable causative agent of cervical cancer in women globally[2,4,14,15]. In this study, we found a high prevalence of HPV antibodies (HPV IgG) in HIV-1 positive women (85.4%). On the other hand, HPV antibodies was the least detected in HIV-1 negative women (14.6%). HPV antibodies were more prevalent in HIV-1 positive women that were older in age (>30 years) compared to the younger women ($\leq$30 years) whose blood samples showed the lesser prevalence of HPV antibodies. This result was in conformity with previous studies that found that the prevalence of HPV antibodies (HPV IgG) in HIV-1 infected women was in the range of 40-70% in the USA, Africa and around the world[4,16-18]. Current data also showed a statistically significant association between HPV16 infection and age ($p = 0.0001$). This significant finding corroborated other studies in which HPV infection among HIV-1 infected women was also associated with age[14,16,19]. There was also a significant association of HPV-16 infection with sexual activity amongst women infected with HIV-1 in this study. This outcome was also confirmed by other studies in which HPV infection in HIV-1 infected women was also associated with sexual activity and other socioeconomic factors[20,21]. In Nigeria, a previous study reported that HPV infection is usually age-specific in women[16]. While this study did not report the prevalence of HPV among women living with HIV/AIDS, the study confirms current findings in which HPV frequency was found to be age-specific in the HIV-1 positive women investigated in this current study. HPV type-16 is a high risk strain of HPV and it was detected in 8.8 % of the patient's whole blood samples analyzed by PCR in this study. This result corroborated to earlier reports in parts of Africa, particularly Zambia and Nigeria, in which high-risk of HPV types were detected in about 10-50% of HIV-infected women[16,18,21]. On the other hand, in Nigeria, the prevalence of high risk HPV types including types 16, 18, 6 and 11 in the general population of women was reported to be 26.30%[16], a rate that was far higher than the prevalence rate of HPV detected in this study by PCR (8.8%). More so, the prevalence of HPV among HIV-1 positive women and HIV-1 negative women in southwest Nigeria, Lagos precisely, was 44.90% and 11.20%, respectively[16]. In the Americas and USA, high frequency of multiple HPV genotypes was also reported among HIV-infected women[17,20,22], thus confirming current findings that HPV is a co-infection that could increase the chances of cervical cancer in HIV-1 infected women and probably other populations. Since women could acquire and develop HPV infections following their first sexual intercourse, especially unprotected and extramarital sexual intercourse[2,4,19], there is therefore need to make the screening and detection of HPV in women of active sexual age, particularly amongst those living with HIV/AIDS in order to reduce the frequency of HPV infection in human populations in Nigeria and globally. Such measures will help to control the spread of HPV infection among the human population while ensuring that people living with HIV/AIDS gets better prognosis after treatment.

CONCLUSION

Women already infected and living with HIV-1 are more at risk of developing cervical cancer due to HPV infection, particularly those caused by the HPV16 strains-which is known to be globally distributed. In conclusion, this preliminary study reported the prevalence of high risk HPV types (HPV type-16) in HIV-1 positive women and thus reiterates the need for location-specific and prompt detection of HPVs in such target group as a way of halting the transmission of HPV infection in human population in Nigeria. It was believed that continuous detection and monitoring of HPV16 amongst HIV-1 infected women and other groups will help in improving patients prognosis and thus slow the development and spread of cervical cancer in HPV16/HIV co-infected women in developing countries and globally.

SIGNIFICANCE STATEMENT

This study highlights the public health risk of HPV infection in HIV-1 positive people and reiterates the need for continuous monitoring and reporting to mitigate the transmission of the disease in human population in Nigeria.

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