



The Seroprevalence of Human Papilloma Virus (HPV) Types 6,11,16 and 18 among Women Attending Cervical Screening (Pap Smear) Service in Abakaliki, Southeastern Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author BAFN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Author GOE managed the analyses of the study, edition of the manuscript and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The seroprevalence of the causative agent of cervical cancer: Human Papilloma Virus (HPV) types in any region is essential for the effective prevention and control of cervical cancer in that region, since there are over 100 types of HPV with high and low risk types. In this study the occurrence of HPV types 6, 11, and 16 & 18 were studied among women attending cervical screening (Pap smear) center in a Federal Teaching Hospital Abakaliki, Ebonyi State Nigeria. Consents were obtained and blood samples were aseptically collected and processed for HPV assay using HPV IgG ELISA kits from Cusabio Co. LTD Germany. The results showed that out of 360 women aged between 20 years and 63 years with a mean age of 34±9 years who came for Pap smear screening,

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only 25% (90) gave consent for HPV testing and participated in the study. The overall result showed that 71.1% (64) were positive and 28.9% (26) were negative. The high risk HPV16 and HPV 18 had the prevalence of 22.2% and 21.2% respectively, while the low risk HPV 6,11, had 27.8%. We concluded that there is higher seroprevalence of low risk HPV types 6,11 than high risk HPV types 16,18; and low acceptance of HPV testing in Abakaliki, southeastern Nigeria (25%). We recommend an urgent Medical education and sensitization of women in this region about the risk factors for HPV transmission and the need to participate in HPV testing services provided by the teaching hospital in order to prevent the transmission of HPV and thereby control cervical cancer.

Keywords: Prevalence; human papilloma virus (HPV); cervical screening; pap smear; Abakaliki; Nigeria.

1. INTRODUCTION

Globally, Cervical cancer is the third most common cancer among women and the seventh most common cancer overall, with 530,000 new cases and 275,000 deaths annually [1]. In Nigeria, it is the second most common female cancer after breast cancer, with an age standardized incidence rate of 34.5 cases per 100,000 women in 2010 [2,3]. About 48,000,000 women are at risk in Nigeria, 17, 550 women are diagnosed yearly, 9, 659 women die annually and 26 women on daily basis from cervical cancer [4].

Cancer is the malignant, uncontrollable growth of cells or tissues resulting from the accumulation of sublethal genetic injuries which transform normal cells to neoplastic ones [5]. It is well established that Human Papilloma Virus (HPV) is the causative agent of cervical cancer [6,7]. Human papilloma Virus (HPV) is a double stranded, non enveloped DNA virus from the Papilloma virus family and usually infect the keratinocytes of skin or mucous membranes of humans. There are more than 100 types of HPV, and they have the capacity to integrate into the host genome. These viruses contain genes encoding for early function (E1-E8), involved in viral DNA replication, transcriptional regulation, and cellular transformation [8,9].

HPV is typically transmitted through sexual contact and infect the anogenital region causing no symptoms in most people. Some, the low risk types may cause genital warts (verrucae), while others especially those with persistent infection with "high-risk" HPV types may progress to precancerous lesions and invasive cancer of the cervix [10,11]. In the pathogenesis of cervical cancer there are two main groups of HPV: the high risk types- 16,18,33,45, and low risk types- 6,11,55,39, they cause cervical cancer and genital warts respectively. Types 16 and 18

account for about 70% of all cases of cervical cancer [12,13].

In a worldwide study of HPV prevalence involving 11 countries including Nigeria by Clifford et al. [13], Nigeria had 25.6% and Spain had 1.4%. They observed that the prevalence of HPV were highest in sub-Saharan Africa. Thomas et al. [14], observed in Ibadan Nigeria that high risk HPV had prevalence of 19.7% while low risk HPV had 6.6%. They also observed other type of HPV such as HPV42, HPV41, HPV 16, and HPV35. In another study in Lagos Nigeria by Nweke et al. [15], the prevalence of HPV among HIV positive women were studied. They observed that the prevalence of HPV among HIV positive women was 44.9% while that in HIV negative women was lower 11.0%. They also observed another difference in the HPV types that occur in HIV positive and HIV negative women. The high risk HPV types observed in HIV positive women were 31, 35, 52, 53, while that in HIV negative women were 16, 18, 52, 56.

As can be observed from the studies in Ibadan, Lagos and 11 countries there is heterogeneity in the occurrence of HPV types among women from different populations even in the same country [14,16-23]. There are few studies on Prevalence of Human Papilloma Virus (HPV) in Nigeria and these few studies were in the western part of the country [13-15]. With the introduction of cervical cancer (HPV) vaccine it becomes imperative that more studies on the prevalence of HPV types are needed in other part of the country, to determine the seroprevalence HPV types and best vaccine for each region.

The objective of this study is to determine the seroprevalence of HPV types, the high risk types 16, and 18; and the low risk types 6, and 11. We also determined the acceptability of HPV testing as part of cervical screening services among

women in Abakaliki, Ebonyi-State, South eastern Nigeria.

2. MATERIALS AND METHODS

The participants in this study attended the cervical screening clinics of Federal Teaching Hospital Abakaliki either as a patient or a participant who wish to be screened, and those who gave their consent were included in the study.

2.1 Sample Collection

After detailed explanation about the study, Questionnaires were administered to each patient/participant before sample collection. The samples were collected using [24,25] methods, after consent has been obtained and questionnaire filled.

2.2 HPV Testing [25]

Blood was drawn aseptically and serum prepared. Samples were diluted: 1:101 into a properly defined dilution tubes, and mixed carefully on vortex. 100 µL of negative and Positive controls and diluted samples were dispensed into properly identified wells in duplicates. The microplates were incubated for 60 min at 37°C. The microplates were washed using an automatic washer. 100 µL of Enzyme conjugate was pipetted into each well, except the A1 well, and covered with the sealer. The microplates were incubated for 60min at 37°C. The microwells were washed again. 100µL chromogen/substrate mixture were pipetted into each well, including the blank well and incubated at room temperature (18-24°C) for 20 min. Samples were read with Microwell reader at 450 nm. The samples were both samples and duplicates were positive were accepted as positive, while those ones in which either the sample or the duplicate is positive is repeated.

2.2.1 Precaution and pre-assay controls

All the components were allowed to reach room temperature about 1hrs before use. The ELISA reader was turned on 20 minutes before reading. No finger prints are present on the bottom of the microwell before reading at 450 nm. Reading was carried out just after the addition of the stop solution. The whole test was not exposed to strong illumination.

2.3 Data Analysis

Data was analysed using SPSS version 16. Cross tabulation, chisquare (χ^2) and correlation statistics were applied.

2.4 Ethical Consideration

Ethical approval was obtained from the hospital ethical committee, (see appendix). The patients results, medical history and human specimen provided were handled with utmost confidentiality and with strict adherence to ethical guidelines and standard regulations [26].

3. RESULTS

360 (100%) participants aged between 20-63years filled the questionnaires, but only 90 (25%) gave consent for HPV assay, (Table 1). There is low up take of HPV testing (25%), in Abakaliki Southeastern Nigeria.

Fig. 1 shows that 71.1% of those who gave consent were positive and 28.9% were negative for HPV assay.

Fig. 2 shows the seroprevalence of the various types of HPV: HPV 16 (22.2%), HPV 18 (21.2%), HPV 6;11 (27.8%). There is higher seroprevalence of low risk HPV types 6,11and lower seroprevalence of high risk HPV types 16;18.

4. DISCUSSION

The results showed that out of 360 women aged between 20 years and 63 years with a mean age of 34 ± 9 years who came for Pap smear screening, only 25% (90) did HPV testing. There has been general poor up take of HPV testing in the southeastern part of Nigeria, [27,28]. In a study by Nwozor and Oragudosi [27] in Onitsha southeast Nigeria only 1.8% of studied population had done pap smear or HPV test. Chinaka and Nwazue [29], in a study of the awareness of cervical cancer and its screening in Abakaliki Nigeria also observed that it is only11.1% that had cervical screening in their study. Similar observations were recorded by Eze et al. [30], and Igwilo [31], Olusegun and Adepiti [32]. However making known the benefit of screening and HPV testing to the participants can greatly increase the willingness to participate in the screening [33].

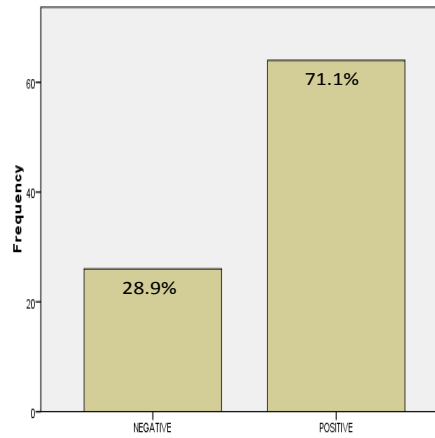


Fig. 1. Seroprevalence of hpv among women in abakaliki

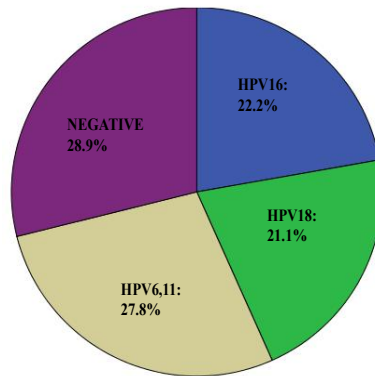


Fig. 2. Seroprevalence of hpv types

Table 1. Age group and hpv status of the respondent

Age group (years)	HPV status of the respondent				Total
	No consent yet	Negative	Positive	Total consent	
20-29	Count 63 % 63.6%	8 8.1%	28 28.3%	36 36.4%	99 100.0%
30-39	Count 127 % 75.1%	15 8.9%	27 16.0%	42 24.9%	169 100.0%
40-49	Count 51 % 81.0%	3 4.8%	9 14.3%	12 19.1%	63 100.0%
50-59	Count 22 % 100.0%	0 .0%	0 .0%	0 .0%	22 100.0%
60-69	Count 7 % 100.0%	0 .0%	0 .0%	0 .0%	7 100.0%
Total	Count 270 % 75.0%	26 7.2%	64 17.8%	90 25%	360 100.0%

N =360, Mean = 34±9, Mode 30, Median 34, Range 20-63 years

It was also observed that the older the women the less willing to have HPV assay since percentage of total consent was decreasing as the age of the women were increasing. The participants who were aged 50 years and above did not give consent for the study, (Table1).

The HPV assay results showed that 71.1% (64) were positive and 28.9% (25) were negative, (Fig. 1). The result showed high seroprevalence of HPV in our environment. This observation is critical in view of the fact that this is the first time the seroprevalence of HPV types were being studied in our environment and with the background of high incidence of cervical cancer in our environment. In a worldwide study of HPV prevalence involving 11 countries including Nigeria by Clifford et al. [13], Nigeria had 25.6% and Spain had 1.4%, although these were lower than our observations they observed that in overall that HPV were highest in sub-Saharan Africa than other parts of the world studied.

Fig. 2 showed that high risk HPV16 and HPV 18 had the seroprevalence of 22.2% and 21.2% respectively, and HPV 6,11 had 27.8%. This observation means that there is higher seroprevalence of low risk Human Papilloma Virus (HPV6,11) among the participants than the high risk type (HPV16, HPV18), and within the high risk type HPV16 is higher in occurrence than HPV18. Thomas et al. [14], observed in Ibadan Nigeria that high risk HPV had prevalence of 19.7% while low risk HPV had 6.6%. This is a reversal of our observation where low risk HPV had a higher prevalence than high risk HPV. In another study in Lagos Nigeria by Nweke et al. [15], the prevalence of HPV among HIV positive women were studied. They observed that the prevalence of HPV among HIV positive women was 44.9% while that in HIV negative women is lower 11.0%. Their high risk HPV had 37.5%. They also observed another difference in the HPV types that occur in HIV positive and HIV negative women. The high risk HPV types observed in HIV positive women were 31, 35, 52, 53, while that in HIV negative women were 16, 18, 52, 56. The observation in the HIV negative women is similar with our finding.

As can be observed from the studies in Ibadan, Lagos and Abakaliki in this our work that there is heterogeneity in the occurrence of HPV types among women from different populations even in the same country. Similar observation was also made by other studies, [13,19, 21,22]; hence the need to study the seroprevalence of HPV types

in any particular region to effectively prevent HPV transmission and control cervical cancer.

5. CONCLUSION AND RECOMMENDATIONS

There is high seroprevalence of HPV infection among women in Abakaliki, Ebonyi-State, southeastern, Nigeria. These women are mostly infected by low risk HPV types 6, 11, than high risk types 16, and 18. Finally, the uptake of HPV testing among these women was poor.

We recommend urgent and extensive health education of women in the southeastern Nigeria, with emphasis on HPV infections, its risk factors and dangers of cervical cancer which can be prevented. We also recommend more research on the sociocultural factors influencing cervical screening up take in the southeastern Nigeria since older women are reluctant in giving consent.

CONSENT

Informed consent was obtained from all the participants before commencing the study.

ETHICAL APPROVAL

Ethical approval was obtained from the Federal Teaching Hospital ethical committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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