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Prevalence of Human Papillomavirus Deoxyribonucleic Acid in Cervical Cancer Specimens in Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author GII designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors DA, KAO, MAN and OO managed the analyses of the study. Authors UA and EMI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the prevalence of human papillomavirus (HPV) deoxyribonucleic acid in cervical cancer specimens in Calabar, Nigeria.
Study Design: This is a retrospective prevalence, a cross-sectional study on archival cervical cancer specimens.
Place and Duration of Study: This study was done at the department of pathology, University of Calabar Teaching Hospital, Calabar, on cervical cancer specimens between 1st January 2009 and 31st December 2014.

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Methodology: Paraffin-embedded tissue block of all the invasive cervical cancer specimen received during the study period were collected. Basic socio-demographic data were obtained from the medical records. Sections of the tissue were obtained from the blocks, digested using proteinase K solution and the DNA was extracted. A polymerase chain reaction and DNA enzyme immunoassay (DEIA) was done. The results of the DNA enzyme immunoassay were read, and the samples were categorized as HPV DNA positive or negative.
Results: One hundred and twenty-three cervical cancer specimens were analysed. There were one hundred and ninety-six gynaecological malignancy specimens received during the study period, giving an invasive cervical cancer prevalence of 62.7% among the gynaecological malignancies in the centre. The samples were from female subjects aged from 32 to 75 years. Their mean age was 48.6 ± 10.6 (years). A majority (86) which made up 69.9% of the subjects was below 51 years. The peak age group of the disease among the subjects is 42 – 51 years. One hundred and thirteen (91.90%) of these samples were HPV DNA positive while ten (8.10%) of the samples were HPV DNA negative. The prevalence of HPV DNA in the samples by age group distribution shows the highest prevalence of 38.6% from the 42-51 years age group followed by those in a 31 - 40 years age group (33.3%), 61 – 70 years (16.7%), 51 – 60 years (10.5%) and the age group with the lowest prevalence is >70 years (1.6%).
Conclusion: There is a need for the precise pattern of HPV DNA prevalence in cervical cancer in every part of the world to be established. The fill of this knowledge gap would help in enhancing the development of strategies targeted at the elimination of cervical cancer globally.

Keywords: Cervical; cancer; deoxyribonucleic acid; virus; epithelium.

1. INTRODUCTION

Human Papillomavirus (HPV) is a double-stranded deoxyribonucleic acid (DNA) virus that has been implicated in the aetiology of cervical cancer. Human papillomavirus is transmitted between people through skin-to-skin or sexual contact. The human Papillomavirus infect immature cervical squamous epithelium at the stratum germinativum at the squamocolumnar junction. The HPV infect these squamous epithelial cells and integrate their DNA into that of the host DNA, producing viral proteins such as the E6 and E7 proteins which induce a malignant transformation in these cells. E6 achieves its oncogenic effect by inhibiting the activity of P53 while E7 carries out its oncogenic effect by inhibiting the activity of RB. When HPV infect the squamous epithelium, it causes viral cytopathic changes such as koilocytosis (with nuclear atypia and perinuclear halo). These cytopathic changes are found in the premalignant lesions of the cervical epithelium referred to as squamous intraepithelial lesion or cervical intraepithelial neoplasia. In cervical intraepithelial neoplasia, there are atypical changes in the epithelium but with no evidence of breach of the basement membrane. This premalignant lesion then transforms into a malignancy with time. There are two types of human papillomavirus that are capable of causing malignant transformation in the cervical epithelium - low risk and high risk human papillomavirus. The Low-risk types have been occasionally implicated as an aetiological agent in cervical cancer. The high-risk human papillomavirus has been known to be the major aetiological agent of cervical cancer worldwide. There are about 19 high-risk human papillomavirus including types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 and 82. [1,2] The low-risk human papillomavirus include types 6,11, 40, 42, 43, 44, 54, 55, 57, 61, 62, 70, 70, 71, 72, 74, 81, 83, 84 and 89.[1] There are also human papillomavirus of undetermined-risk which include types 2a, 3, 7, 10, 13, 27, 28, 29, 30,34, 86, 87, 90 and 91.[1] The commonest HPV genotypes from a population based study in Turkey were 16, followed by 51, 31, 52 and 18 [3].

Cervical cancer has posed a huge burden of disease on the female population worldwide. More than half a million cases of cervical cancer and over 300,000 deaths from the disease are recorded every year, globally [4]. Papanicolaou test is a screening test for cervical cancer which enables premalignant cervical lesions and cervical cancer to be discovered early by finding abnormal cervical epithelial cell changes when successful treatment is possible. In the United States, deaths from cervical cancer declined by more than 50% in the last 30 years mostly due to the effectiveness of Papanicolaou test for cervical cancer screening [5]. In 2012, a guideline was jointly established by the United States Preventive Services Task Force, the

American Society for Colposcopy and Cervical Pathology, the American Society for Clinical Pathology and the American Cancer Society. They recommended using cytology and HPV co-testing for cervical cancer screening [6,7,8].

The introduction of the vaccines against HPV 16 and 18 has brought the hope of achieving a reasonable reduction in the burden of cervical cancer. There are two available vaccines against HPV 16 and 18 which include the bivalent vaccine Cervarix (HPV 16 and 18 vaccines) and the quadrivalent vaccine Gardasil (vaccinate against HPV 6, 11, 16 and 18). On December 10, 2014, the food and drug administration (FDA) approved the 9-valent HPV vaccine (Gardasil 9) which is targeted against HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 [9]. These vaccines are composed of viral-like proteins produced using recombinant technology. They are capable of stimulating an immunologic response [10]. Cervarix and Gardasil are very effective vaccines against the human papillomavirus 16 and 18. Squamous cell carcinoma and adenocarcinoma are the commonest forms of cervical cancers in Calabar, constituting 97.6% and 2.4% of the cervical cancers respectively [11].

There are several risk factors associated with persistent human papillomavirus infection of the uterine cervical epithelium and the subsequent development of invasive cervical cancer. They include the use of multiple sexual partners, oral contraceptives, immunosuppression, an early debut of sexual intercourse, high parity, low socio-economic status and cigarette smoking [12,13,14].

2. METHODOLOGY

2.1 Study Design and Materials

The study design is a prevalence study on archival specimens at the University of Calabar Teaching Hospital (UCTH), Calabar. The cervical cancer cases diagnosed between 2009 and 2014 was identified. The paraffin-embedded tissue blocks of cervical cancer were selected. Basic information (e.g. age at diagnosis and year of diagnosis) was collected from the medical records.

2.2 Sample Size

The sample size for this study consists of all histological samples of cervical cancer seen in UCTH between 1st of January 2009 and 31st December 2014.

2.3 Paraffin Block Processing

Two sections are obtained from each paraffin block. The sections of the blocks were kept in Eppendorf tubes. The specimens were processed under strict conditions to avoid potential contamination. A tissue-free paraffin block is cut after processing each study block to detect any HPV carry-over from block to block. For each block, a new blade is used and the microtome is cleaned with Histoclear II and 70% alcohol. To further control possible sources of contamination paraffin seven blocks containing non-HPV related lesions processed at the same time as the cervical cancer specimens in the pathology laboratory were blindly included in the process. These specimens were labelled as controls.

2.4 HPV DNA Detection

The tissue sections obtained were deparaffinized in xylene and centrifuged at 16,000g (14,000 rpm) for 3 minutes. This is done twice. The tissue is rehydrated by addition of graded concentration of ethanol including absolute ethanol, 70% ethanol and 50% ethanol serially in that order. The sample is then centrifuged at 14,000 rpm for 3 minutes and the supernatant decanted. The tissue was digested using freshly prepared Proteinase K solution which was added and incubated overnight at 56 ^{o} C. Additional proteinase K solution was added several times to ensure digestion of the tissue. After the proteinase K solution digestion, the DNA is extracted in phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1). First, buffered phenol was added, then it was centrifuged and the top aqueous layer was transferred to a new tube. The phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1) were added subsequently and the above process as with buffered phenol was repeated. The top aqueous layer which contains both DNA and RNA was transferred to a new tube. RNAse was added and incubated at 37°C for 1hour to digest the RNA. The DNA extraction in phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1) is repeated as above to separate the DNA from the RNA and RNAse.

After DNA extraction, ethanol precipitation of the DNA is done. First 1/10 volume of sodium acetate and 2.5 times volume of 100% ethanol was added to the extract. It was then incubated at 20°C for 30 minutes and centrifuged at 14,000

rpm for 30 minutes at 4°C. DNA pellets then precipitated out of the solution. The supernatant was aspirated and the DNA pellet was washed with 70% ethanol and centrifuged at 14,000 rpm for 10 minutes. The supernatant was aspirated and the DNA pellet was dissolved in distilled water.

10 µl of the extracted DNA was used to perform an SPF-10 Polymerase chain reaction(PCR) using SPF-10 primers that target the 65 base pair region of the HPV L_1 open reading frame. This enables the amplification of at least 54 HPV types. A deoxyribonucleic acid (DNA) enzyme immunoassay (DEIA) was performed on the amplified PCR products using probe hybridization with a cocktail of conservative probes that can recognize 54 mucosal HPV genotype in a microtiter plate format for HPV DNA detection. The reverse primers contain biotin label at the 5' end, enabling the capture of the reverse strand into the streptavidin-coated microtiter plates. Captured amplimers were then denatured by alkaline treatment and detected by a defined cocktail of digoxigenin-labelled probes, allowing the detection of at least 54 HPV genotypes. The optical densities (OD450) were read on a microtiter plate reader and the samples were categorized as HPV DNA positive or negative.

2.5 Data Analysis

Data were entered and analyzed using Epi Info7 software, with descriptive and inferential statistics employed for analysis. Frequency tables, graphs and charts were used to display sociodemographic characteristics as well as the prevalence of HPV DNA among specimens in the study period. Alpha level of significance was set at 0.05.

2.6 Criteria for Selection

Blocks of paraffin embedded tissue specimen diagnosed of invasive cervical carcinoma during the study period (between 1st January 2009 and 31st December 2014).

2.6.1 Exclusion criteria

Cases in which the tissue blocks are missing and cases diagnosed by Pap smear cytology were excluded from this study. Nine specimens were excluded from this study because their blocks were missing.

2.7 Ethical Consideration

Ethical clearance for the conduct of this study was obtained from the relevant authorities.

3. RESULTS

3.1 General Findings

One hundred and twenty-three cervical cancer specimens were analyzed in this study. The specimens were received at the department of pathology, University of Calabar Teaching Hospital, Calabar. There were one hundred and ninety-six gynaecological malignancy specimens received during the study period, giving a cervical cancer prevalence of 62.7% among the gynaecological malignancies in the centre.

3.2 Sociodemographic Characteristics of Subjects

A total of 123 female subjects aged from 32 to 75 years were studied. Their mean age was 48.6 ± 10.6. Table 1 shows the age groups of subjects. Majority 86(69.9%) was aged below 51 years while the least number of subjects 2(1.6%) comprised of those aged above 70 years.

Table 1 shows the peak age group of cervical cancer in the study to be 41 – 50 years with a prevalence of 37.4%, followed by 31 – 40 years (32.5%), 61 – 70 years (17.1%), then 51 – 60 years and the age group with the least prevalence is >70 years(1.6%). The mean age in this study is 48.6±10.6 years.

3.3 Results of HPV Testing

The one hundred and twenty-three specimens were analyzed for human papillomavirus (HPV). One hundred and thirteen (91.90%) of these samples were HPV DNA positive while ten (8.10%) of the specimens were HPV DNA negative.

Fig. 1 shows the result of the samples that were analyzed. Out of a total of 123 samples that were analyzed, 113(91.9%) were positive for HPV DNA, thus giving an overall prevalence of HPV infection in the cervical cancer specimens of 91.9%.

Fig. 2 shows the prevalence of HPV DNA in the specimens by age group distribution. The age group 41-50 years has the highest prevalence of

38.6%, followed by those in a 31 - 40 years (33.3%), 61 – 70 years (16.7%), then 51 – 60 years (10.5%) and the age group >70 years has the least prevalence (0.9%).

Table 1. Showing the age distribution of the subjects

Age group (Years)	Frequency (N=123)	Percentage (%)
31-40	40	32.5
41-50	46	37.4
51-60	14	11.4
61-70	21	17.1
>70	2	1.6
Mean Age ± SD	48.6±10.6	

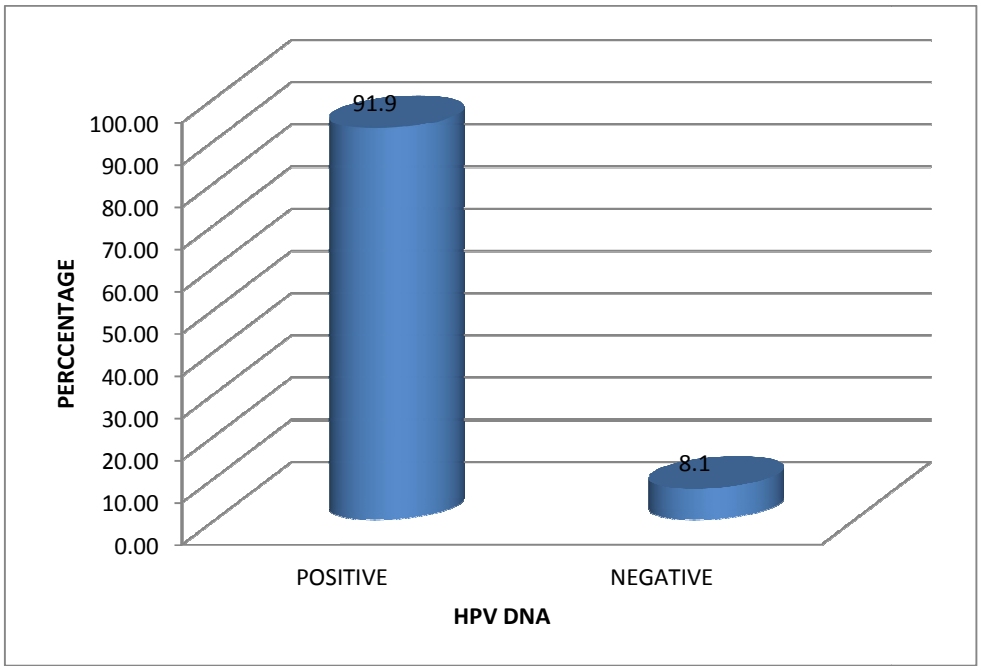

Fig. 1. Showing prevalence of HPV DNA (human papillomavirus deoxyribonucleic acid)

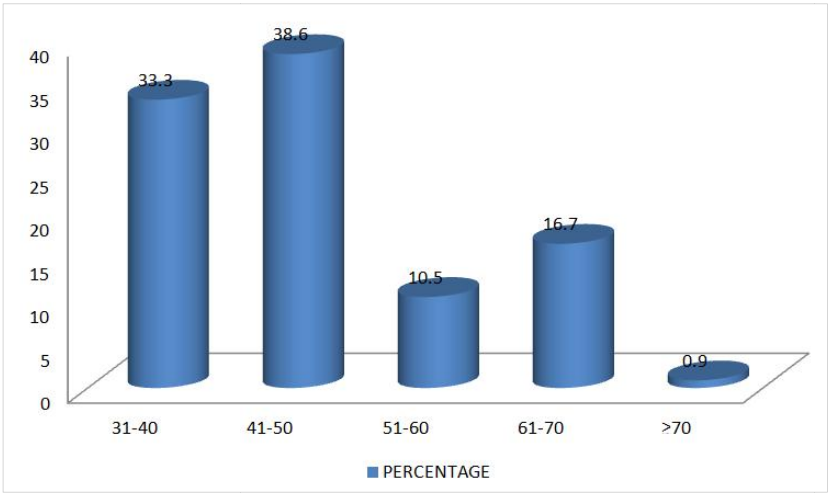

Fig. 2. Prevalence of HPV DNA by age distribution

Table 2. Relationship between HPV DNA status and age group of study samples

Age group (Years)	HPV DNA Positive (%)	HPV DNA Negative (%)	Total	Chi-square	p-value
31-40	38(33.6)	2 (20)	40	20.0	0.2
41-50	42 (37.2)	3(30)	45	df=16	
51-60	12 (10.6)	2 (20)	14		
61-70	19 (16.8)	2 (20)	21		
>70	1 (0.9)	1 (10)	2		
Total	113(100)	10(100)	123		

Table 2 shows the relationship between HPV DNA status and age group of participants. The age group 41 – 50 years has the highest prevalence among the HPV positive specimen(37.2%), followed by 31 – 40 years(33.6%), 61 – 70 years (16.8%), 51 – 60 years (10.6%) and then >70 years (0.9%). Subjects in the 31-40 years age group were more likely to have HPV infection with a prevalence of 95.0% as compared to other age groups. However, this relation was not statistically significant. X^2= (16, N=123) = 20.00, *p*= 0.2).

4. DISCUSSION

Most cervical cancer cases result from genital infection with human papillomavirus (HPV). Well-organized programmes of regular gynaecological screening and treatment of precancerous lesions have been effective in preventing squamous cell cervical cancer (the most common) but have had less impact on adenocarcinoma of the cervix. Gynaecological screening programmes are also difficult to implement in low-resource settings. However, vaccines targeted against HPV genotypes causing cervical cancer have the potential of eliminating the disease.

One hundred and thirty-two cervical cancer specimens were received in the department of pathology, University of Calabar Teaching Hospital during the six-year study period. Nine specimens were excluded from the study because the tissue blocks were missing. This makes it one hundred and twenty-three specimens that were analyzed in this study. This represented 62.7% of the entire specimens diagnosed with gynaecological malignancy during this study period. This finding is consistent with that from a similar study by Ekanem et al. which shows a prevalence of 63% [15]. This value is, however, lower than that obtained by Pindiga et al. and Mohammed et al. which found a prevalence of 72.6% and 77% respectively. [15, 16,17,18,19] This difference could be due to a relatively early age marriage of females in the northern part of the country (Nigeria) associated with the early debut of sexual intercourse.

The mean age of the women in this study of 48.6 ±10.6 years is consistent with findings in Zaria, Nigeria, by Sule et al. with a mean age of 47.6 years, in Zimbabwe by Ndlovu et al with a mean age of 48 years and other parts of the world [20,21]. A similarly study by Der et al in Ghana of women with cervical cancer show a mean age of 57.8 years which is in agreement with that from this study [22]. The age range of the women in this study is between 32 and 75years with peak age group 41-50 years. This peak age group is in agreement with findings from studies in Nigeria by Ekanem et al., Omotoso et al., Mohammed et al., Pindiga et al., Ijaiya et al. and in other parts of Africa by Mushosho et al. [15,17,23,24]. These similarities could be due to the similarities in the culture/lifestyle and environmental factors in sub-Saharan Africa.

The prevalence of HPV DNA in the study specimen is 91.9%. A similar study done in Ibadan, Nigeria, by Okolo et al. showed the prevalence of HPV DNA in invasive cervical cancer to be 90.7% which is in agreement with that from this study [25]. Another study that was done by Denny et al. Sub-Saharan Africa involving Nigeria, Ghana and South Africa found a HPV DNA prevalence in invasive cervical cancer of 84.9%, 93.9% and 92.1% respectively [26]. Though this finding is generally consistent with that from this study, the prevalence for Nigeria in this study by Denny et al is slightly lower than that from this study. Also, a study by Boumba et al. in the Democratic Republic of Congo shows an HPV DNA prevalence of 98.3% for invasive cervical cancer specimen which is higher than that obtained from this study [27]. Rossi et al. in a similar study in Italy found a HPV DNA positivity prevalence of 96% which is also higher than that from this study [28]. The worldwide prevalence of HPV DNA in invasive cervical cancer is 99.7% and an

underestimation of HPV prevalence in cervical cancer is most likely due to the limitations of study methodologies [29,30]. The slightly lower prevalence of HPV DNA in invasive cervical cancer specimens in this study as compared to those obtainable worldwide may be as a result of a low viral load in some specimen (concentration of HPV DNA below detectable level) and technical artifacts due to poor DNA quality.

In this study, the age distribution of HPV DNA positive cases shows highest prevalence at age 41-50 years which represent 37.2% of the total HPV DNA positive cases. This is followed by age 31 – 40 years with a prevalence of 33.3%, followed by age 61-70 years (16.8%), then 41-50 years (10.6%) and the least prevalent age group is >70 years with a prevalence of 0.9%. The finding that age group 41-50 years having the highest proportion of the overall HPV DNA positive cases is not surprising since it was also the age group with the highest cervical cancer prevalence in a study by Irabor et al. [11]. A study by Parkin et al. in Netherlands on the prevalence of high-risk HPV infection among women and the prevalence of cervical cancer showed that both cervical cancer and high-risk HPV infection was highest between ages 20 to 40 years [31]. However, in this study, the age group 31 – 40 years has the second highest prevalence of HPV infection in the specimens following the age group 41 – 50 years. According to a publication from the Bulletin of the World Health Organization from a worldwide study done by Franceschi et al., there is an inverse relationship between age and human papillomavirus (HPV) prevalence in many countries. But in some of the poorest areas studied, HPV prevalence was high across all age groups and the age-standardized HPV prevalence varied more than 10 fold between populations [32,33]. These differences and similarities noted may be related to the pattern of sexual activity within each population.

The proportion of HPV DNA positive cases in the individual age group is highest at age 31- 40 years with a prevalence of 95.0% followed by age 41-50 years with a prevalence of 93.3%. The age group with the least proportion of HPV DNA positive cases is age >70 years (50.0%). These findings were not statistically significant. A similar study in India by Streedevi et al. showed the highest prevalence of HPV DNA positivity of invasive cervical cancer in the age group 26-35 years, this finding is consistent with that from this study [34]. These similarities could be due to similarities in the socioeconomic status of most people in both countries.

There is a need for the precise pattern of prevalence of high-risk HPV DNA in cervical cancer in every part of the world to be established. The fill of this knowledge gap would help in enhancing the development of strategies for elimination of cervical cancer globally. Fortunately, with the advent of the 9-valent HPV vaccine which was developed to prevent infection and disease from the nine HPV types (HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, HPV-58) known to cause approximately 90% of cervical cancers and 90% of anogenital warts, we expect that the incidence of cervical cancer would be significantly reduced with time [35,36,37,38,39, 40,41,42,43,44].

5. CONCLUSION

This study has shown a high rate of HPV infection in cervical cancer specimens in this region. Therefore, the introduction of the 9-valent HPV vaccines would hopefully go a long way in reducing the incidence of this disease in this region.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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