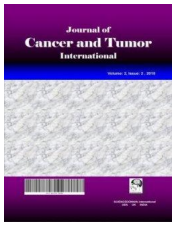

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Specific Human Papillomavirus Genotypes Isolated from Cervical Cancer Specimens in Calabar, Nigeria

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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, EA, KAO and UA managed the analyses of the study. Authors AJO and MAN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine specific human papillomavirus genotypes isolated from cervical cancer specimens in Calabar, Nigeria.
Study Design: This is a cross-sectional study of archival paraffin-embedded tissue block of invasive cervical cancer specimen.
Study Place and Period: This study was done at the department of pathology, University of Calabar Teaching Hospital, Calabar for cervical cancer specimen between January 2006 and December 2014.
Methodology: Paraffin-embedded tissue block of invasive cervical cancer specimen from the study period (January 2006 to December 2014) were collected. Primary socio-demographic data were obtained from medical records in the department (such as surgical pathology register,

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histopathology request forms). Sections of the tissue were obtained from the blocks. The deoxyribonucleic acid (DNA) was extracted and a polymerase chain reaction was done. Then DNA enzyme immunoassay and reverse hybridisation line probe assay were performed for human papillomavirus DNA detection and specific HPV genotyping.

Results: Two hundred and forty-two cervical cancer specimens were analysed. The age range of the subjects is between 32 and 78 years with a mean age of 47.4 years. Two hundred and twenty-one (91.30%) of these samples were HPV DNA positive. Seven high-risk HPV (hrHPV) genotypes were isolated from these specimens which include types 16, 18, 31, 33, 35, 59 and 68/73.

The prevalence of the specific HPV genotype in invasive cervical cancer specimens are as follows: type 16 has the highest prevalence of 34%, followed by type 31 with a prevalence of 31.2%, type 18 with a prevalence of 16.3%, type 33(8.3%), Type 35(7.7%), Type 59(4.1%) and type 68/73 has the least prevalence of 1.8%. Multiple infections represented 4.10% of all the HPV DNA positive samples. Various infections with types 16 and 31 have a prevalence of 2.7% while multiple infections with types 16 and 35 have a prevalence of 1.4%.

Conclusion: The knowledge of the prevalent high-risk HPV in cervical cancer in our environment would enhance the development of a more appropriate and effective vaccine against HPV involved in the aetiology of cervical cancer.

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

1. INTRODUCTION

Human papillomavirus (HPV) infections are the most frequent sexually transmitted infections in the United States of America. About 14 million new genital HPV infections occur each year.[1] The Center for Disease Control and Prevention estimates that more than 80%, of sexually active women, will be infected with at least one type of HPV at some point in their lives. [2]

The human papillomaviruses (HPV) are non-enveloped viruses which possess icosahedral symmetry, 72 capsomers and double-stranded circular DNA genome with the nucleus as its site of replication. The viral genome is divided into an early region, which is necessary for neoplastic/malignant transformation; a late region, which codes for the capsid proteins; and a regulatory region which contains the origin of replication and control elements for transformation and replication [3,4]. There are eight open reading frames (E1 to E8) in the early region and two in the late part (L1 and L2), all of which are located on the same strand of DNA [3]. The oncogenic HPV is responsible for this malignant transformation in cervical epithelium. The virus preferably infects immature squamous epithelium of the stratum basalis. This is seen following erosion of the cervical epithelium that leads to the expose of immature cells of the basal layer and from squamous metaplasia at the squamocolumnar junction of the cervix. When it infects the cell, the virus integrates its DNA into that of the cell and begins to produce oncoproteins E5, E6, E7, but E6 and E7 are usually responsible for the malignant transformation.[5-7] E6 Oncoprotein binds to p53 and stimulates ubiquitin-dependent proteolytic degradation of p53, thus interrupting the death pathway. E6 also up-regulates telomerase, preventing replicative senescence. Also, E7 binds to hypophosphorylated Rb protein, promoting its proteolytic degradation thus allowing E2F to stimulate transcription freely. E6 and E7 induce centromere duplication and genomic instability.[5,7,8] The cytopathic changes found in the premalignant lesions of the cervical epithelium caused by the HPV infection are referred to as squamous intraepithelial neoplasia or cervical intraepithelial neoplasia.[7] In cervical intraepithelial neoplasia, there are apparent atypic changes in the epithelium but with no evidence of a breach of the basement membrane. Within 2 years of acquiring the infection, it is usually cleared in most persons. It is persistent infection by these oncogenic viruses that result in the initial premalignant lesions and then malignant transformation of the infected cells.[6] Several factors have been associated with persistent infection. These include Multiple sexual partners, high parity, cigarette smoking, low socioeconomic status, immunosuppression, use of oral contraceptives and young age at first sexual intercourse.

Human papillomavirus has been shown to be the most important aetiologic agent in the development of cervical carcinoma.[9] Every year there are 530,000 new cases of cervical cancer and an estimated 273,000 deaths from the disease worldwide making it the second most

common cause of malignancy in females following breast cancer but the commonest of gynaecological malignancy.[3,4,10] There are variations in the incidence and mortality of cervical cancer from country to country.[10] Eighty percent of women with this disease is in the developing countries with the developing countries having two to three times higher mortality compared to those in developed countries.[7] Cervical cancer is the most prevalent cause of cancer-related death in Central America, South America, south-central Asia, west, east and central Africa.[7] The highest incidence of cervical cancer death has been observed in Guinea.[4,10] Cervical cancer can occur in females between age 18 and 80 years. The peak age of the disease is between 44 and 49 years.[3]

2. MATERIALS AND METHODS

2.1 Study Design and Materials

The study design is a prevalence study on archival specimens. The cervical cancer cases diagnosed between January 2006 and December 2014 was identified. The paraffin-embedded tissue blocks of cervical cancer were selected. Basic information (age at diagnosis, year of diagnosis and original histopathological diagnosis) was collected from medical records.

2.2 Sample Size

The sample size for this study consists of all histological samples of cervical cancer in the University of Calabar Teaching Hospital, Calabar (UCTH) between January 2006 and December 2014.

2.3 Pathology and Laboratory Procedures

Paraffin blocks processing- At least four paraffin sections are obtained for each block. The first and last sections were used for histopathological evaluation after Hematoxylin and Eosin (H&E) staining to confirm the diagnosis of invasive cervical cancer is done. The in-between sections of the blocks are kept in Eppendorf tubes for HPV/DNA testing. Paraffin-embedded blocks 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. To further control possible sources of contamination paraffin twelve blocks containing non-HPV related lesions were labelled as controls and also processed.

HPV DNA detection and genotyping: The tissue sections obtained were deparaffinised in xylene and centrifuged at 14,000 rpm for 3 minutes. The tissue is rehydrated by addition of graded concentration of ethanol. After ethanol rehydration, the tissue was digested using freshly prepared Proteinase K solution which was added and incubated overnight at 56ºC. Additional proteinase K solution is added several times to ensure digestion of the tissue. After the proteinase K solution digestion, DNA is then extracted in phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1). This involves the first addition of buffered phenol, then it was centrifuged and the top aqueous layer was transferred to a new tube. The top aqueous layer which contains both DNA and RNA is transferred to a new tube. Then RNAse is added and incubated at 37ºC for 1 hour 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 2.5times volume of 100% ethanol was added to the extract. It was then incubated at -20ºC for 30 minutes and centrifuged at 14,000 rpm for 30 minutes at 4ºC. DNA pellets precipitated out of the solution. The supernatant was aspirated and the DNA pellet was washed with 70% ethanol and centrifuged for 10 minutes. The supernatant was aspirated and the DNA pellet dissolved in distilled water.

10 µl of the extracted DNA was used to perform an SPF-10 Polymerase chain reaction 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 a probe hybridization with a cocktail of conservative probes that can recognise at least 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 categorised as HPV DNA positive or negative. The HPV DNA positive samples were subsequently analysed by $LiPA_{25}$, a reverse hybridization line probe assay technique that detects 25 high-risk and low-risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, 74). Specimens that were HPV DNA positive but did not hybridize with any of the 25 probes are to be coded as HPV type X (undetermined type). The SPF-10 amplimers were used to identify HPV genotype by reverse hybridization to the $LiPA_{25}$ genotyping strip.The Positive hybridization on the strips is visualized as a purple band by means of a precipitating colour substrate on the probe site. Because the interprimer regions for HPV 68 and 73 are identical, both genotypes cannot be distinguished by this test. Specimens that were positive for this probe were reported as 68/73.

2.4 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 and prevalence of cervical cancer, as well as prevalence and genotypes of HPV among subjects in the study period. Alpha level of significance was set at 0.05.

2.5 Criteria for Selection

Blocks of paraffin-embedded tissue specimen diagnosed with invasive cervical carcinoma during the study period (January 2006 to December 2014).

2.6 Exclusion Criteria

Cases in which the tissue blocks are missing and cases diagnosed by Pap smear cytology were excluded from this study. Thirteen 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 health research ethics committee of the University of Calabar Teaching Hospital, Calabar with the protocol assigned number UCTH/HREC/33/268.

3. RESULTS

3.1 General Findings

For the nine-year study period (January 2006 to December 2014), two hundred and forty-two cervical cancer specimens were analyzed of the total samples received in the department of pathology, University Of Calabar Teaching Hospital, Calabar during the study period. There were three hundred and eighty-seven gynaecological malignancy specimens received during this period, giving a cervical cancer prevalence of 62.5% among the gynaecological malignancies in the centre.

3.2 Sociodemographic Characteristics of Subjects

A total of 242 female subjects aged from 32 to 78 years were studied. Their mean age was 47.4 ± 5.5. Table 1 shows the age groups of subjects. Majority 169 (69.8%) were aged below 51 years while the least number 5 (2.1%) comprised of those aged above 70 years.

Table 1. Showing the age distribution of the subjects

Age group (Years)	Frequency (N=242)	Percentage (%)
31-40	79	32.6
41-50	90	37.2
51-60	27	11.2
61-70	41	16.9
>70	5	2.1
Mean Age ± SD	47.4±5.5	

3.3 Results of HPV DNA Detection and Genotype

Two hundred and forty-two sample specimens were analyzed for Human Papillomavirus (HPV) from within this nine years study period. Two hundred and twenty-one (91.3%) of these specimen were HPV DNA positive while twenty-one (8.7%) of the samples were HPV DNA negative. Seven high-risk HPV (hrHPV) types were isolated from these specimens including Types 16, 18, 31, 33, 35, 59 and 68/73.

221(91.3%) were positive for HPV DNA, thus giving an overall prevalence of HPV infection among cervical cancer specimens of 91.3% and 8.7% of the samples were negative for HPV DNA.

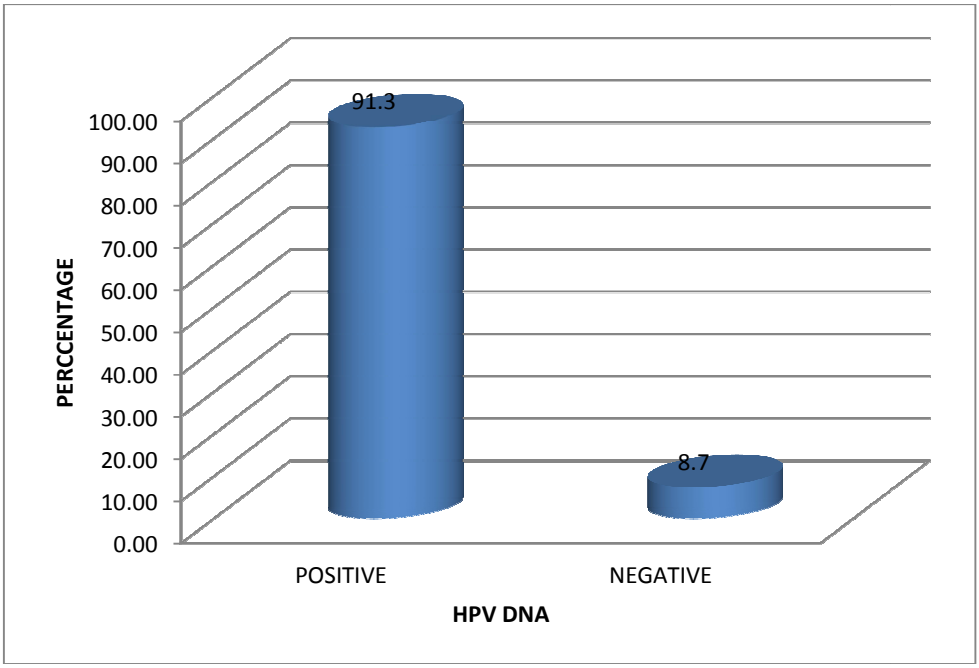

Fig. 1. Showing prevalence of HPV DNA (human papillomavirus deoxyribonucleic acid)

Table 2. Prevalence of specific HPV genotype in the cervical cancer specimens

HPV genotype	Frequency (N=221)	Prevalence (%)
TYPE 16	66	34.0
TYPE 31	63	31.2
TYPE 18	36	16.3
TYPE 33	20	8.3
TYPE 35	14	7.7
TYPE 59	9	4.1
TYPE 68/73	4	1.8
TYPES 16 and 31	6	2.7
TYPES 16 and 35	3	1.4

Table 2 shows the prevalence of HPV genotype in invasive cervical carcinoma among the study samples. Type 16 has the highest prevalence of 34.0%, followed by type 31 with a prevalence of 31.2%, type 18 with a prevalence of 16.3%, type 33 is 8.3%, Type 35 is 7.7%, Type 59 is 4.1% and type 68/73 has the least prevalence of 1.8%. Multiple infections with types 16 and 31 have a prevalence of 2.7% while multiple infections with types 16 and 35 have a prevalence of 1.4%.

Table 3 shows the relationship between histological type and HPV status among study subjects. Positive status among subjects was more likely to be non-keratinizing squamous cell carcinoma 143(91.1%) although not statistically significant; no statistically significant relationship was found between the histological types of cervical carcinoma and the HPV DNA status of the subjects, X^2 = (3, N=242) =5.6, p= 0.1

Table 3. Relationship between histological types and HPV status

Histological type	HPV DNA status			Statistics	
	Positive N=221(%)	Negative N=21(%)	Total N=242	Chi-square	p-value
ADC	4(66.7)	2 (33.3)	6	5.6	0.1
BSCC	5 (71.4)	2 (28.6)	7	df=3	
KSCC	69 (95.8)	3 (4.2)	72		
NSCC	143(91.1)	14 (8.9)	157		
Total	221 (91.3)	21 (8.7)	242		

ADC = Adenocarcinoma; BSCC = Basaloid Squamous Cell Carcinoma;
KSCC = Keratinizing Squamous Cell Carcinoma; NSCC = Non-Keratinizing Squamous Cell Carcinoma

4. DISCUSSION

Cervical cancer represents a huge percentage of gynaecological malignancies worldwide being the commonest gynaecological malignancy.

A total of two hundred and forty-two specimens were analyzed in this study. Cervical cancer represented 62.5% of all the specimen 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%. [11] This value is, however, lower than that obtained by Mohammed et al which found a prevalence of 77% respectively. This difference could be due to a relative earlier age of marriage amongst the females in the northern part of the country (Nigeria) [12].

In this study is 47.4 ± 5.6 years is mean age of the women which is consistent with findings in Zaria, Nigeria by Sule et al with a mean age of 47.6 years, and Der et al in Ghana for women with cervical cancer show a mean age of 57.8years.[13,14] The age range of the women in this study is between 32 and 78 years with the peak incidence at the 41-50 years age group. This age group of peak incidence is in agreement with findings in studies in Nigeria by Omotoso et al, Irabor et al, Mohammed et al, Ijaiya et al and Mushosho et al.[12,15-17] These similarities may be as a result of similarities in the socio-cultural lifestyle and environmental factors in sub-Saharan Africa.

From this study, 69.8% of cervical cancer specimens are from women whose ages are ≤ 48years. A similar finding was observed by Mohammed et al in a study in Zaria which showed that 57.98% of the cases of cervical cancer occurred in females that are ≤49 years.[12] However, a study by Der et al in Ghana shows that 70% of the women were above 50 years, which are in contrast to the findings from this study.[14] The peak age of cervical cancer worldwide is 45 years.[4] Cervical cancer is the fourth most common cancer in women worldwide and the second most common female cancer in women aged 15 - 44 years old worldwide.[18] This would generally explain why more women ≤48 years had cervical cancer in this study.

The HPV DNA prevalence in this study is 91.3%. One study that was done in Ibadan, Nigeria by Okolo et al showed the HPV DNA prevalence of 90.7% in invasive cervical cancer, which is similar to that from this study.[19] A study that was done by Denny et al in Nigeria, found an HPV DNA prevalence in cervical cancer of 84.9%.[20] This finding is slightly lower than that from this study. Also, a study by Rossi in Italy and Boumba et al in the Democratic Republic of Congo shows an HPV DNA prevalence of 96% and 98.3% respectively which is higher than that from this study [21,22]. 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.[23] The slightly lower prevalence of HPV DNA in invasive cervical specimens in this study as compared to those obtainable worldwide may be as a result of a low viral load in some specimen and technical artefacts due to a poor quality of HPV DNA.

Human Papillomavirus genotype identified in this study are high-risk HPV genotype. There was seven high-risk HPV genotype identified in this study including types 16, 18, 31, 33, 35, 59, 68/73. Because the interprimer regions for HPV 68 and 73 are identical, both genotypes cannot be distinguished by this test. Specimens that were positive for this probe were reported as 68/73.

In this study, 95.9% of the HPV DNA positive specimen had single HPV infection and 4.1% of cases had multiple HPV infections. Ndiaye et al in their study showed that prevalence of cases with multiple infections among cervical cancer specimen to be 3.6% which is consistent with that obtained in this study.[24] Another study by Coutlee et al in Canada showed that multiple HPV infections were demonstrated in 20.2% of invasive cervical cancer which is higher than that obtained in this study.[25] This could be due to a lower viral load of some of the HPV types present in the tissue making them difficult to detect by the methodology used.

The commonest HPV genotype identified in this study is type 16 with a prevalence of 34.0%. This finding is consistent with that from a similar study in Ibadan, Nigeria by Okolo et al in which HPV 16 was the most common HPV genotype identified and constituted 67.6% of HPV positive cases of invasive cervical cancer which is higher than that from this study.[19] This finding is also consistent with findings from a similar study in Democratic Rebuplic of Congo among Congolese women by Boumba et al where HPV

16 was the commonest HPV type identified from invasive cervical cancer specimen. In this study by Boumba et al, HPV 16 prevalence in invasive cervical cancer was 52.5% which is also higher than that obtained from this study.[22] A study by Ndiaye et al in sub-Saharan Africa, show that the most prevalent HPV genotype identified are types 16, 45, 18, 33 and 35 with a prevalence of 42%, 14.5%, 13%, 4.3% and 4.3% respectively.[24] Another study in Italy by Rossi et al also showed HPV 16 as the commonest HPV type isolated from cervical cancer specimen.[21] However, in this study, all the 9 cases with multiple HPV infections had HPV 16 infection. The difference in the prevalence of HPV 16 could be due to differences in environmental factors which affect the survival and transmission of the virus in the different environments.

The second commonest HPV type identified in this study is type 31. The prevalence of HPV 31 in this study is 31.2%. HPV type 31 is among the commonest HPV identified in invasive cervical carcinoma worldwide.[25] In Africa, HPV 31 is known to be the 5th commonest HPV isolated from invasive cervical cancer specimen. In the North, Central, South America and Australia, HPV 31 is the third commonest HPV type identified in invasive cervical cancer, and in Europe, it is the fourth commonest and worldwide HPV 31 is the fourth commonest HPV identified in invasive cervical cancer specimen.[7] In the study by Okolo et al in Ibadan, it was the seventh commonest HPV genotype identified in invasive cervical cancer specimen in the study constituting 1.5% of HPV positive cases which is much lower than what was obtained in this study.[19] In this study, HPV 31 is identified in 80% of cases with multiple HPV infections. The difference in the prevalence of HPV 31 could also be due to differences in environmental factors which affect the survival and transmission of the virus in the different environments.

The third commonest HPV genotype identified in this study is type 18 with a prevalence of 16.3%. This prevalence is consistent with the worldwide prevalence of HPV 18 in invasive cervical cancer of 14.9% - 16.2%.[7] Worldwide, it is the 2nd most prevalent HPV type identified in invasive cervical cancer specimen.[7] In the study by Okolo et al, HPV 18 was the second most prevalent HPV genotype (10.3%) identified among invasive cervical cancer specimen, though the prevalence is slightly lower than that from this study (16.3%).[19] A similar study by Ndiaye et al in sub-Saharan Africa revealed that HPV 18 was the 3rd commonest HPV genotype identified in cervical cancer specimen with a prevalence of 13%.[24] Also, the worldwide study by Bosch et al shows that HPV 18 constituted 10 - 20% of HPV genotype identified in invasive cervical cancer and precancerous lesions of the cervix.[26] The difference in the prevalence of HPV 18 could be due to differences in environmental factors which affect the survival and transmission of the virus in the different environments.

The fourth commonest HPV type isolated in this study is HPV 33 with a prevalence of 8.3% followed by type 35 (7.7%), type 59(4.1%) and then type 68/73 with a prevalence of 1.8%. [7] In the study by Ndiaye et al in 2012 in sub-Saharan Africa, HPV 33 was also the fourth commonest HPV genotype identified in the cervical cancer specimen with a prevalence of 4.6%.[24] This prevalence is lower than that obtained from this study.[24] HPV 33 is the fifth commonest type of HPV identified in cervical cancer worldwide and the fourth commonest in Africa. This study also shows that HPV 35 had the fifth commonest genotype with a prevalence of 6.3%. A study by Santos et al in 2001 in Peru show an HPV 35 prevalence of 4.2% which was the 5th commonest HPV genotype isolated from the cervical cancer specimens.[27] The difference in the prevalence of HPV 33 and 35 could also be due to differences in environmental factors which affect the survival and transmission of the virus in the different environments.

Multiple HPV infections were observed in 9 out of the 221 HPV DNA positive cases which constitute 4.1% of the HPV DNA positive specimens. However, the study by Coutlee et al in Canada revealed multiple HPV infections in 20.2% of invasive cervical cancer which is higher than that obtained in this study.[28] This could be due to differences in the technique/methodology of HPV DNA extraction or variability in the viral load in the tissue.

There was no statistically significant correlation between HPV DNA status and histological type of cervical cancer. The relationship between HPV DNA status and histological type shows that non-keratinizing squamous cell carcinoma has the highest prevalence (64.7%) among the HPV DNA positive specimen followed by keratinizing squamous cell carcinoma (31.2%), followed by basaloid squamous cell carcinoma (2.3%) and

adenocarcinoma had the least prevalence (1.8%) among the HPV DNA positive samples. For each histological type, keratinizing squamous cell carcinoma has the highest proportion of HPV DNA positive cases (95.8%), followed by non-keratinizing squamous cell carcinoma (91.1%), basaloid squamous cell carcinoma (75.0%) then adenocarcinoma (66.67%) which has the least proportion of HPV DNA positive cases.

The available HPV vaccine for some time has been those effective against types 6, 11, 16 and 18, type 16 and 18 being the high-risk type of HPV. A study by Chinchai et al in 2012 show a prevalence of HPV 16 and 18 in cervical cancer specimens to be 71% which is higher than that from this study.[29] But, recently the FDA has approved the 9-valent human papillomavirus vaccine, Gardasil 9 which is effective against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58.[30] The CDC has recommended the administration of this vaccine to be administered children from 9 years of age.[31] HPV 16 and 18 together are known to cause about 70% of invasive cervical cancer worldwide.[32-42] In this study, both HPV 16, 18, 31 and 33 together were identified in 89.8% of cases positive for HPV DNA. The obvious deduction from this is that 89.8% of those cases positive for HPV DNA could be prevented by the 9-valent HPV vaccine in this environment. A vaccine targeted against the four commonest HPV genotype identified in cervical cancer from this study (types 16, 18, 31 and 33) would be capable of preventing 92% of HPV DNA positive cases in this study. Similarly, a vaccine that would target the five commonest HPV type identified in this study (types 16, 18, 31, 33 and 35) would be capable of preventing 98.3% of HPV DNA positive invasive cervical cancers in this region. However, there is no concrete evidence to support that this 9-valent HPV vaccine is capable of preventing cervical cancer caused by the other high-risk HPV.[43-50].

5. CONCLUSION

The seven HPV type identified in invasive cervical cancer in this study are type 16, 31, 18, 33, 35, 59 and 68/73 in order of prevalence. Similar research needs to be done in different regions of the world to improve the knowledge of the prevalent types of HPV causing invasive cervical cancer. This would enhance a more strategic approach in the fight against cervical cancer.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

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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