Prevalence and Distribution of High Risk Human Papillomavirus Subtypes in Invasive Cervical Cancer in South-West Nigeria

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Abstract

Context: Despite the high incidence of cervical cancer in Nigeria, there are only a few large scale population based studies on HPV prevalence and genotype distribution. **Aims:** This study was carried out to determine the prevalence and distribution of human papillomavirus (HPV) types in invasive cervical cancer (ICC) in Nigeria. **Settings and Design:** Cross sectional period prevalence study. **Methods and Material:** Paraffin embedded tissue blocks of two hundred and thirty five archival cervical cancers diagnosed in three participating laboratories from South West Nigeria were analyzed for the presence of HPV DNA. Statistical Analysis: Data analysis was performed with the Statistical Package STATA 10. **Results:** Of the one hundred and eighty seven samples that were considered appropriate for HPV detection after histological evaluation 160 (85.6%) were positive for HPV DNA. The five most common types identified as single types among HPV positive cases were HPV16 (46.9%), HPV18 (19.4%), HPV45 (11.9%), HPV35 (5.0%), and HPV31 (3.1%). Others were HPV33, 39, 51, 52, 56, 58, 59, 66, and 68. HPV16 and 18 in single/multiple infections accounted for 69.4% of the samples. Multiple infections were detected in 4.4%. All the adenosquamous and neuroendocrine carcinomas tested positive for HPV, while 86.1% and 66.7% of the squamous cell and the adenocarcinomas were positive, respectively. **Conclusion:** These results are in consonance with reports from all other parts of the world that HPV16 and 18 accounts for almost 70% of cervical cancers, supporting data that effective vaccination against these two types will reduce the cervical burden in South West Nigeria.

Keywords: Cervical cancer, human papillomavirus DNA, human papillomavirus

INTRODUCTION

In the 90s, the human papillomavirus (HPV) was designated an oncogenic virus following the demonstration of its DNA in cervical cancer from many countries around the world.[1,2] Over hundred HPV types have been identified of which forty infect the genital tract primarily through sexual transmission. Of the many serotypes of HPV DNA identified, those of HPV types 16 and 18 were found to be predominate though studies have shown some variation in the serotypes among different populations.[2,3] Despite, the high incidence of cervical cancer in Nigeria, there are only a few large-scale population-based studies on HPV prevalence and genotype distribution reported from this region and none in archival cervical cancer samples.[4-6] The aim of this study is to elucidate the prevalence of high-risk HPV as well as to elucidate the types found in archival specimen from cervical cancer cases diagnosed in some centers from the South-West of Nigeria between 2000–2004.

This study is a part of a larger study by the RIS HPV TT study group whose objective was to describe the HPV genotype distribution in (ICC) worldwide.

MATERIALS AND METHODS

The study design was a cross-sectional period-prevalence study on archival specimens. Paraffin-embedded tissue blocks of ICC were consecutively selected from the three participating laboratories in South-West Nigeria. Cervical cancer cases diagnosed between 2000 and 2004 were identified. Basic information (age at diagnosis, year of diagnosis, and original histopathological diagnosis) were also collected from the medical records.

Paraffin blocks processing

Blocks were reembedded wherever necessary. At least four paraffin sections were obtained for each block (sandwich method). First and last sections were used for histopathological

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evaluation after hematoxylin and eosin (H and E) staining. The in-between sections of the blocks were 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 was cut after processing each study block to prevent any HPV carryover from block to block. For each block, a new blade was used and the microtome was cleaned with Histoclear II and 70% alcohol. To further control for possible sources of contamination, the paraffin blocks containing nonHPV-related lesions processed at the same time as the cervical cancer specimens in the local pathology lab were blindly included in the process at a ratio of 5% per ICC samples. These specimens were labeled as controls.

Histopathological evaluation

The paraffin blocks processing and the pathology reassessment of the histopathological diagnosis was done by the reference pathology laboratory (Institut Català d'Oncologia (ICO), Barcelona and was performed following the consensus criteria established by an expert panel of pathologists based on the World Health Organization classification of the uterine cervix tumors.[7] The pathology evaluation included: diagnosis of histological type (ICC: squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, other types; noninvasive cervical cancer and control tissue specimen); the presence of mucosa or preneoplastic lesions adjacent to ICC (Cervical intraepithelial neoplasia-CIN1/2/3; Adenocarcinoma *insitu* (AIS)); degree of necrosis; amount of tumor infiltration; and adequacy of the sample for further HPV testing. A block was determined to be adequate for HPV testing, if invasive cancer was observed in the two H and E stained sections of the specimen. In case of discrepancies between the local and the reference laboratories, the results obtained at the reference lab prevailed.

Human papillomavirus/DNA detection and typing

This was done using the methodology outlined by Kleter *et al*.[8] 250 μl of freshly prepared Proteinase K solution was used to extract the DNA. SPF-10 PCR was performed using 10 μl of the DNA extract in a final reaction volume of 50 μl. All samples were run with a 1:10 dilution. The amplified PCR products were tested using a probe hybridization with a cocktail of conservative probes recognizing, at least, fifty four mucosal HPV genotypes in a microtiter plate format for the detection of HPV/DNA. Optical densities (OD450) were read on a microtitre plate reader. HPV/DNA positive samples were subsequently analyzed by HPV SPF-10 LIPA$_{25}$ (version 1: Labo Biomedical Products, Rijswijk, Netherlands), a reverse hybridization technique that detects twenty five 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, 70, and 74). The sequence variation within the SPF-10 primers allows the recognition of these different HPV genotypes, except for types 68 and 73, as their interprimer regions are identical and cannot be distinguished by this test. After PCR, 10 μl of the amplimers were used to perform reverse hybridization for HPV genotype identification. Positive hybridization on the strips is visualized as a purple band by means of a precipitating color substrate on the probe site. Specimens that were HPV/DNA positive but did not hybridize with any of the twenty five probes were coded as HPV type X (uncharacterized type). SPF-10 LIPA$_{25}$ PCR detection and typing analysis were performed at ICO.

Statistical analysis

Data analysis was performed with the Statistical Package STATA 10. Overall, HPV positive and HPV type-specific proportions were determined. 95% Confidence Intervals (95% CIs) for proportions were estimated. To estimate the HPV type distribution, single and multiple HPV infections were considered either separately or combined. A stratified analysis of the overall HPV positivity by age at diagnosis and histological characteristics was performed. Age at diagnosis was recoded as a categorical variable (age: ≤39, 40–49, 50–59, and ≥60 years).

Ethical issues

Specimens were received anonymously (without name and/or original medical record number) at the reference laboratory in Barcelona (ICO). All protocols were approved by international and ICO ethics committees and all the study progress was overseen by an international steering committee specifically formed for the supervision and advising in critical issues of the project.

RESULTS

There were two hundred and thirty five histologically diagnosed carcinomas of the cervix seen during the period of study. Of the 187 samples considered appropriate for HPV detection after histological evaluation, there were 173 squamous cell carcinomas, 9 adenocarcinomas, 2 adenosquamous carcinomas and 3 neuroendocrine carcinomas. Data on age were available for 155 cases, and the age range was from 22 to 86 years with an average age incidence of 55.1yrs. The number of cases increased from the 3rd decade with a peak in the 6th decade.

One-hundred and sixty cases (85.6%) were positive for HPV DNA [Table 1]. Among the HPV DNA positive cases, a single HPV type was identified in 151 cases (94.4%; 95% CI: 90.8%–97.9%), type X in two cases (1.3%; 95% CI: 0%–3.0%), and multiple types in seven cases (4.4%; 95% CI: 1.2%–7.5%). The five most common single types among positive cases were: HPV16 (46.9%), HPV18 (19.4%), HPV45 (11.9%), HPV35 (5.0%), and HPV31 (3.1%) [Table 2]. In all seven multiple infections, two HPV types (double infections) were identified, five of which had type 16 in addition to another. All cases with multiple infections were squamous cell carcinomas. HPV16/HPV18 (HPV16 and 18 as single types plus HPV16and18) accounted for a 66.9% (95% CI: 59.6%–74.2%) of the HPV positive ICC cases, and this percentage reached 69.4% (95% CI: 62.2%–76.5%) when multiple infections of HPV 16 with another high-risk type were counted.

HPV16 was the common type identified in squamous and adenocarcinoma cases, although there was more HPV18 in

Table 1: Detection of human papillomavirus/DNA in invasive cervical cancer cases from Nigeria, by age at diagnosis and histopathological information

|	Analysed cases (n)	HPV positive cases (n)	HPV detection Percentage (95% CI)
Age (years)			
≤39	13	14	92.9 (79.4–100)
40–49	26	32	81.3 (67.7–94.8)
50–59	45	53	84.9 (75.3–94.5)
≥60	50	56	89.3 (81.2–97.4)
Missing information	26	32	81.3 (67.7–94.8)
Histological type			
Squamous cell carcinoma	149	173	86.1 (81.0–91.3)
Adenocarcinoma	6	9	66.7 (35.9–97.5)
Adenosquamous carcinoma	2	2	100 (-)
Other types (neuroendocrine)	3	3	100 (-)
Degree of necrosis (%)			
<25	139	158	88 (82.9–93)
25–50	14	20	70 (49.9–90.1)
51–75	5	7	71.4 (38.0–100)
76–90	2	2	100 (-)
Tumour infiltration (%)			
<10	5	7	71.4 (38–100)
10–25	24	28	85.7 (72.8–98.7)
26–50	54	62	87.1 (78.8–95.4)
51–75	55	63	87.3 (79.1–95.5)
>75	22	27	81.5 (66.8–96.1)
Pre-neoplastic lesions adjacent to the invasive cervical cancer			
Yes	16	19	84.2 (67.8–100.6)
No	144	168	85.7 (80.4–91.0)
Total	160	187	85.6 (80.5–90.6)

HPV: Human papillomavirus, CI: Confidence interval

adenocarcinomas than HPV 16 [Figure 1]. It was identified either in single/multiple infection in 51.0% and 50.0% of the squamous and adenocarcinomas, respectively. HPV18 was found in two cases of neuroendocrine carcinomas, while HPV35 accounted for one case of neuroendocrine carcinoma. Both cases of adenosquamous carcinoma had HPV16 and 45.

Discussion

HPV was detected in 160, of the 187 cervical cancer cases analyzed giving an overall prevalence rate of 85.6%. This is high when compared the findings from Uganda by Odida *et al.* but low when compared to those of de Cremoux *et al.* and Peedicayil *et al.* who recorded prevalence rates of 96 and 95% from Italy and India, respectively.[9-11] The lower prevalence from our studies may be attributable to use of unbuffered formalin for fixation of tissues in the selected laboratories.

Most of the infections were due to specific HPV genotypes with mixed infections occurring in only 4.4% of cases. This is similar to the findings of Odida *et al.* from Uganda and el-All *et al.* from Egypt, who also reported an association with Bilharzial infection.[9,12] It is however low compared to the findings from Italy, France, and Zimbabwe where mixed infections were found in 18, 22.2, and 24% of cases, respectively.[13-15] Both
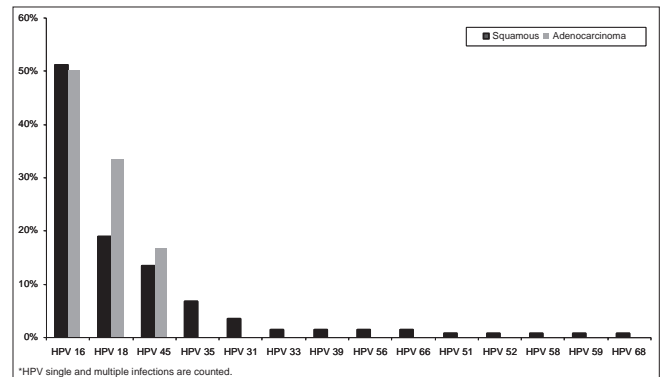
Figure 1: Distribution of human papillomavirus types by histological diagnosis (squamous cell carcinoma and adenocarcinoma) in invasive cervical cancer specimens from Nigeria. *Human papillomavirus single and multiple infections are counted

types 16 and 18 were found in ≥50% of the mixed infections. Differing data on the frequency of coinfections with multiple HPV types detected in cervical neoplasia have been attributed to the typing methodology and population studied. Sebbelov *et al.* found that infections with multiple genotypes were prevalent in Alaskan (36.5%) but not in Greenland natives (3.7%) and Danish caucasians (6.9%).[16] Multiple infections have been found to have an impact on treatment outcomes. Munagala *et al.*

Table 2: Distribution of human papillomavirus types in invasive cervical cancer specimens from Nigeria

	HPV-type specific positive cases (*n*)	HPV-type specific relative contribution (*n*=160) Percentage (95% CI)
Single types	151	94.4 (90.8–97.9)
HPV 16	75	46.9 (39.1–54.6)
HPV 18	31	19.4 (13.3–25.5)
HPV 45	19	11.9 (6.9–16.9)
HPV 35	8	5.0 (1.6–8.4)
HPV 31	5	3.1 (0.4–5.8)
HPV 33	2	1.3 (0.0–3.0)
HPV 39	2	1.3 (0.0–3.0)
HPV 56	2	1.3 (0.0–3.0)
HPV 66	2	1.3 (0.0–3.0)
HPV 51	1	0.6 (0.0–1.8)
HPV 52	1	0.6 (0.0–1.8)
HPV 58	1	0.6 (0.0–1.8)
HPV 59	1	0.6 (0.0–1.8)
HPV 68	1	0.6 (0.0–1.8)
Multiple types	7	4.4 (1.2–7.5)
HPV 16 and 45	2	1.3 (0.0–3.0)
HPV 16 and 18	1	0.6 (0.0–1.8)
HPV 16 and 35	1	0.6 (0.0–1.8)
HPV 16 and 51	1	0.6 (0.0–1.8)
HPV 35 and 45	1	0.6 (0.0–1.8)
HPV 35 and 51	1	0.6 (0.0–1.8)
Type X (uncharacterized type)	2	1.3 (0.0–3.0)
Potential vaccine impact of vaccinated types		
HPV16, 18, 16 and 18	107	66.9 (59.6–74.2)
HPV16, 18, 16 and 18, 16 and 45, 16 and 35, 16 and 51	111	69.4 (62.2–76.5)

HPV: Human papillomavirus, CI: Confidence interval

in their study on the significance of multiple HPV infection in cervical cancer patients and its impact on treatment response found multiple infections to be particularly associated with HPV types 58 and/or 18. These were associated with the risk of treatment failure and therefore had to be followed up for response and suitable interventions done for a favorable outcome.[17] Multiple infections have also been found to be associated with increased cervical cancer risk.[18]

Regarding HPV type distribution, the recent reviews have confirmed that HPV16 is consistently the most common type of HPV in cervical cancer and HPV18 the 2nd common with some minor geographical differences.[19] This corresponds with a recently concluded metanalysis which found HPV types 16 and 18 to be the most common among African women.[20]

Studies have demonstrated a predominance of HPV16 in invasive squamous cell carcinomas and HPV18 in adenocarcinoma though the proportions may vary.[8,14] This was not the finding in this study, where HPV16 was still the dominant type in the adenocarcinomas (50.0%) with both types 18 and 45 equally represented. A study in Colombia, South America attributed 93% of adenocarcinoma cases to HPV types 16, 18, and 45.[21]

Two of the neuroendocrine carcinomas identified were positive for type 18 while the 3rd was positive for type 35. This finding is similar to the findings of Wang *et al*. and Ishida *et al*. who also demonstrated a prevalence of HPV type 18 in their studies on the clinicopathological features of neuroendocrine carcinoma.[22,23] Grayson *et al*. and Parwani *et al*. however found type 16 predominating particularly in the unusual types of neuroendocrine carcinoma.[24,25]

Specific types of HPV were not identified in two cases (type X). Similar findings have been reported from Uganda where there were five cases that could not be specified.[9] The authors raised the possibility of unidentified HPV types being present in the sample.

Studies from the Northern part of Nigeria also confirm the high prevalence of HPV types 16 and 18 in cervical cancer cases.

Conclusion

The prevalence of HPV DNA in archival samples of cervical cancer from South-West Nigeria is 85.6% supporting its role in the etiopathogenesis of cervical cancer in this region.

HPV16 and 18 are the common types and account for about 70% of cases supporting the fact that vaccination against these two types will prevent at least 70% of ICC. A 3rd of the invasive cancers are not associated with HPV16/18 indicating that screening for cervical neoplasia should be maintained after prophylactic vaccination against these types.

HPV 18, while found to be dominant in the neuroendocrine carcinomas is not the dominant type in the adenocarcinomas as reported from most parts of the world. HPV type 45 is highly prevalent in invasive carcinoma and therefore it is recommended that future vaccines should be tailored according to local HPV type distribution.[26]

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Conflicts of interest
There are no conflicts of interest.

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