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Human papillomavirus distribution in invasive cervical carcinoma in sub-Saharan Africa: could HIV explain the differences?

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Abstract

OBJECTIVES To describe human papillomavirus (HPV) distribution in invasive cervical carcinoma (ICC) from Mali and Senegal and to compare type-specific relative contribution among sub-Saharan African (SSA) countries.

METHODS A multicentric study was conducted to collect paraffin-embedded blocks of ICC. Polymerase chain reaction, DNA enzyme immunoassay and line probe assay were performed for HPV detection and genotyping. Data from SSA (Mozambique, Nigeria and Uganda) and 35 other countries were compared.

RESULTS One hundred and sixty-four ICC cases from Mali and Senegal were tested from which 138 were positive (adjusted prevalence = 86.8%; 95% CI = 79.7–91.7%). HPV16 and HPV18 accounted for 57.2% of infections and HPV45 for 16.7%. In SSA countries, HPV16 was less frequent than in the rest of the world (49.4% *vs*. 62.6%; $P < 0.0001$) but HPV18 and HPV45 were two times more frequent (19.3% *vs*. 9.4%; $P < 0.0001$ and 10.3% *vs*. 5.6%; $P < 0.0001$, respectively). There was an ecological correlation between HIV prevalence and the increase of HPV18 and the decrease of HPV45 in ICC in SSA ($P = 0.037$ for both).

CONCLUSION HPV16/18/45 accounted for two-thirds of the HPV types found in invasive cervical cancer in Mali and Senegal. Our results suggest that HIV may play a role in the underlying HPV18 and HPV45 contribution to cervical cancer, but further studies are needed to confirm this correlation.

keywords human papillomavirus, genotype distribution, cervical cancer, HIV, sub-Saharan Africa

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide and the causal factor of several malignant tumours (Koutsky 1997; Aral 1999; Cates 1999; Burchell *et al.* 2006; Bruni *et al.* 2010). The role of oncogenic HPV in the development of virtually all invasive cervical cancers (ICC) has been well established (Walboomers *et al.* 1999; Muñoz *et al.* 2003).

Cervical cancer is a major public health problem. It is the second most common cancer among women worldwide with over half a million incident cases and over 275 000 deaths in 2008 (Ferlay *et al.* 2010). In sub-Saharan Africa (SSA), cervical cancer is the most common cancer in women with an estimated 75 000 new cases and 50 000 deaths each year (Ferlay *et al.* 2010). The numbers of new cases and deaths are projected to be respectively 140 000 and more than 95 000 in 2030, if current population statistics and prevention policies remain the same (Ferlay *et al.* 2010).

The incidence of cervical cancer in SSA countries is one of the highest in the world with an overall age-standardised incidence rate estimated at 31 per 100 000 women-year, though it varies by region with 34.5 in Eastern Africa, 33.7 in Western Africa, 26.8 in Southern Africa and 23 in Central Africa (Ferlay *et al.* 2010). In contrast, this rate is 6.6 in Northern Africa and 10.6 in Europe (Ferlay *et al.* 2010).

In Mali and Senegal, an estimated population of respectively 3.38 and 3.20 million women aged 15 years and older are at risk of developing cervical cancer (WHO/ICO 2010). In both countries, 12% of cytologically normal women are estimated to have cervical HPV infection at a given time (WHO/ICO 2010). The standardised incidence rate of cervical cancer is 37.3 and 34.7 per 100 000 women-years and the standardised mortality rate is 28.4 and 25.5 per 100 000 women-years, respectively, in Mali and Senegal (Ferlay *et al.* 2010).

Cervical cancer screening and vaccine coverage in both countries is limited. In Mali, coverage of screening is 7.6% in urban women and 3.4% in rural women aged 18–69 years (WHS 2003); vaccine is not yet offered. Comparatively, in Senegal, screening coverage is estimated to be 7.4% in urban women and 6.9% in rural women (WHS 2003). Vaccine is available but no national introduction program has been implemented, and most women face financial and access barriers in the private health sector.

Higher incidence rates reported in SSA may be explained by a higher HIV prevalence in these regions. HIV infection has been strongly associated with higher prevalence, incidence and persistence of HPV infection and progression of squamous intraepithelial lesions (De Vuyst *et al.* 2008). More importantly, ICC was classified as an AIDS-defining disease by the Centers for Disease Control and Prevention (1993).

Despite the overwhelming burden estimates, prevention of HPV-related diseases is rarely considered a priority at the decision-making level compared with other well-known infectious diseases that affect the continent such as HIV/AIDS, tuberculosis, malaria and schistosomiasis. Several factors may contribute to the lack of awareness of such preventable disease burden. Provision of direct country-based data on HPV types in cervical cancer may increase knowledge and provide tools for prevention strategies in the region.

Consequently, we conducted our study in two West African countries (Mali and Senegal) to investigate the distribution of HPV genotypes and histopathology in ICC. We also investigated the differences in HPV type distribution in ICC between Mali, Senegal, Mozambique, Nigeria, Uganda and the rest of the world and analyzed the correlation between HIV prevalence and the relative contribution (RC) of specific HPV types in SSA.

We projected this analysis to better comprehend HPV genotypic profile to predict the likely impact of prophylactic HPV vaccination programs as well as to provide important information for the purpose of planning public health needs. This is especially relevant as the GAVI Alliance has prioritised HPV immunisation on its agenda and will support the introduction of the vaccine in GAVI eligible countries starting in 2013 (GAVI 2012).

Materials and methods

Study design and materials

The project was initiated and coordinated by the Catalan Institute of Oncology (ICO) in Barcelona, Spain. We designed a retrospective cross-sectional study to estimate the HPV type distribution in ICC in Mali and in Senegal. Participant centers were:

- *Centre Hospitalier Universitaire A. Le Dantec*, the teaching hospital affiliated with Cheikh Anta Diop University in Dakar; it is a public institution and a reference in the fight against cancer.
- *Hôpital Principal de Dakar*, a military public hospital.
- *Hôpital Général de Grand Yoff*, another major public hospital.
- The pathology laboratory at Cheikh Anta Diop University.
- *Centre Hospitalier Universitaire du Point G*, the teaching hospital affiliated with the University of Bamako.

Patients selected through these sites were representative of the population as the vast majority of cancer patients are diagnosed at these hospitals and referred to their pathology departments and cytology laboratories. Study centers provided available consecutive cases diagnosed with ICC between 2006 and 2010 and information on age at diagnosis, year of diagnosis and original histological diagnosis. Cases were selected using patient registries, which included the pathology report; then all paraffin-embedded blocks from a biopsy or a surgical piece for the same patient were collected from the archives.

Data from Mozambique, Nigeria, Uganda and other regions of the world were obtained from an updated database of the worldwide study of de Sanjosé *et al.* (2010). Since the publication of the latter study, additional ICC cases from Uganda (n = 177) were also available for the present study. Cases were diagnosed between 1968 and 1992 in Uganda, in August and October 1999 in Mozambique and between 2000 and 2004 in Nigeria.

Pathology and laboratory procedures

Upon arrival at the laboratory in ICO, Barcelona, quality of the blocks was assessed based on their external appearance to determine whether the blocks need to be re-embedded. Paraffin blocks were processed under strict conditions to avoid contamination. Four paraffin sections were systematically obtained for each block (sandwich method). First and last sections were used for histopathological evaluation after Haematoxylin and Eosin staining and intermediate sections for HPV DNA detection and genotyping.

Processing and pathology diagnosis were carried out at ICO. Pathology evaluation included: confirmation of ICC, histological type, presence and quantification of tumour necrosis and the percentage of tumour in the whole tissue section and adequacy of the sample for further HPV DNA testing. In between specimens, a blank paraffin section was cut and processed to assess any carry over contamination, in addition to the routine controls of the technique.

For each specimen, a paraffin tissue section was treated with 250 μl of freshly prepared Proteinase K solution to extract DNA. SPF-10 polymerase chain reaction (PCR) was performed using 10 μl of a 1:10 dilution of the crude DNA extract in a final reaction volume of 50 μl. The amplified PCR products were tested using a probe hybridisation step with a cocktail of conservative probes that can recognise around 54 mucosal HPV genotypes using a microtiter plate format for the detection of HPV DNA through a DNA enzyme immunoassay (DEIA) (produced by DDL, Voorburg, the Netherlands). Optical densities (OD450) were read on a microtiter plate reader and categorised as HPV DNA negative, positive or borderline. After PCR, 10 μl of the DEIA HPV DNA positive amplimers were used to perform the reverse hybridisation line probe assay ($LiPA_{25}$) (version 1: produced by DDL). The $LiPA_{25}$ 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, 70, and 74). Positive hybridisation on the strips was visualised as a purple band by means of a precipitating colour substrate on the probe site. Undetermined HPV types (HPV X) were cases which were DEIA positive and $LiPA_{25}$ negative.

Amplification of the human tubulin gene was performed to determine the quality of the DEIA negative samples. Therefore, DEIA positive samples were genotyped with the $LiPA_{25}$ system and the DEIA negative samples were tested for tubulin. DEIA negative and tubulin positive samples were considered negative for HPV while DEIA negative and tubulin negative samples were discarded from the analysis.

Statistical analyses

Overall and type-specific HPV detection percentages and 95% confidence intervals were determined according to country, histology, age and year of diagnosis. Unconditional logistic regression analysis was used to evaluate HPV detection for a given variable taking into account other relevant variables. HPV type-specific RC indicated the proportion of positive cases for a specific HPV type among all HPV positive cases.

Comparisons of HPV16/18/45 RC between SSA countries and other regions of the world were made by using contingency tables and computing the chi-square test of association. Ecological HIV prevalence for each SSA country was retrieved from UNAIDS 2009 report. Estimates and Spearman correlations between HIV and RC of HPV16/18/45 in ICC were calculated. Statistical significance for all of the analyses was set at the two-sided 0.05 level. Data analyses were performed with the statistical package SPSS version 20.0 and STATA version 11.0.

Ethical approvals

All protocols were approved by the appropriate Ethics Committees in all countries where data were collected, and by the Ethics Committees of ICO (Barcelona, Spain) and Sainte-Justine Hospital Research Center (Montreal, Canada). Quality of the study was overseen by an international steering committee.

Results

Sample collection and testing

A total of 204 ICC cases were identified in Mali and Senegal and corresponding blocks retrieved. However, after histopathological evaluation and tubulin testing, the final number of blocks eligible for the final HPV statistical analysis was 164. Figure 1 displays the algorithm of the final sample analysis by country.

Human papillomavirus prevalence and type-specific distribution in invasive cervical carcinoma in Mali and Senegal

Table 1 shows the number of cases included by country, period of diagnosis, age at diagnosis and histological group. The mean age of cases was 51.9 years (SD ± 12.3). Eighty-six cases were diagnosed between 2006 and 2008 and 78 cases in 2009 and 2010. Histopathological classification was as follows: 91.5% squamous cell carcinoma (SCC), 3% adenocarcinoma, 3% adenosquamous

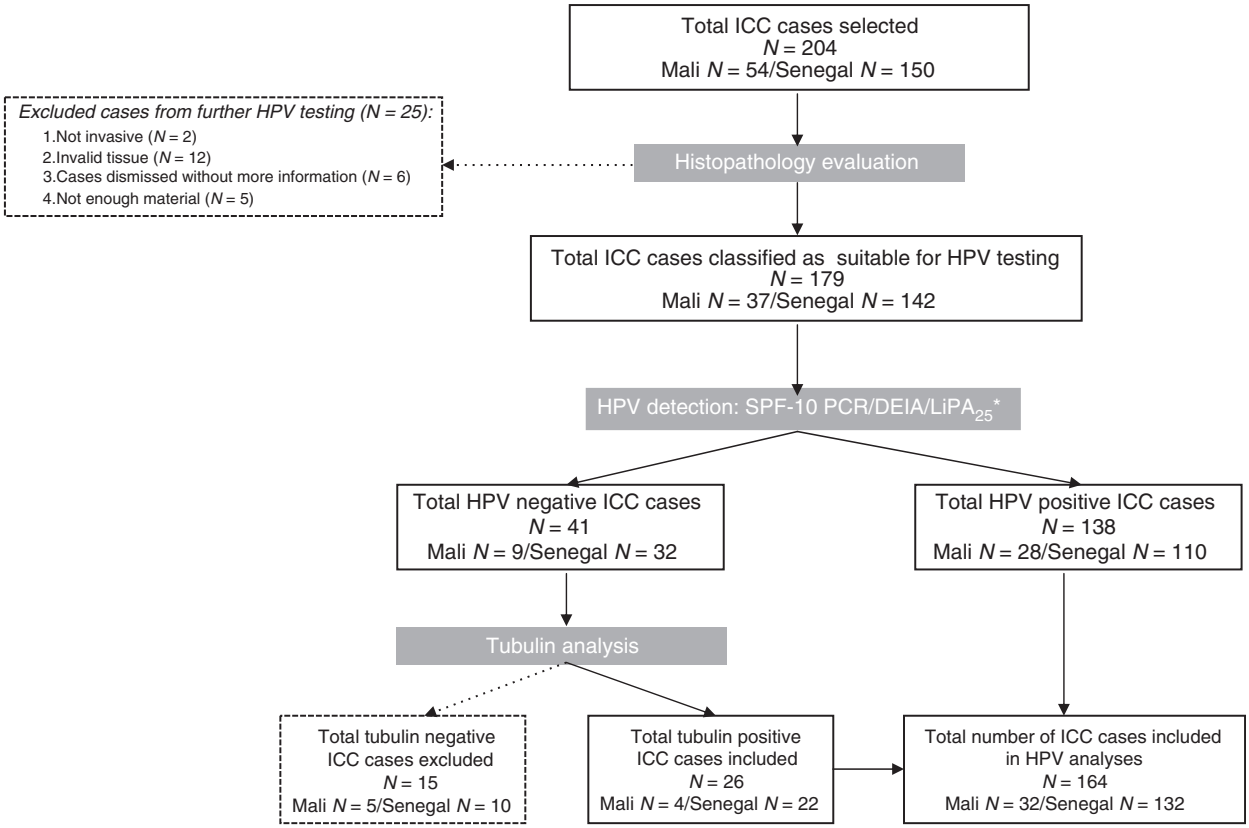

Figure 1 Algorithm of cervical cancer cases included in Mali and Senegal. HPV, human papillomavirus; PCR, polymerase chain reaction; DEIA, DNA enzyme immunoassay; $LiPA_{25}$, line probe assay; ICC: Invasive Cervical Cancer. *$LiPA_{25}$ performed only in DEIA positive cases.

carcinoma and 2.5% were classified as others (three undifferentiated and one neuroendocrine carcinoma).

Overall, 138 of 164 ICC cases tested for HPV were positive. The adjusted prevalence of HPV was 86.8% (95% CI = 79.7–91.7%): 88.1% in Mali (95% CI = 71.3–95.7%) and 86.5% in Senegal (95% CI = 78.5–91.8%). Positivity was higher in older ICC cases (2006–2008) than in more recent cases (2009–2010). Detection of HPV was higher in SCCs when compared with other histologies. However, none of these observations were statistically significant.

Table 2 shows the specifics of HPV distribution among HPV-positive women diagnosed with ICC. A high proportion of single infections were identified (92%; n = 127). The most frequent HPV types detected in decreasing order were: HPV16 (42.0%), HPV45 (14.5%), HPV18 (13.0%), followed by HPV33 and HPV35 (both 4.3%). HPV16 and HPV18 accounted together for 57.2% of all infections. Multiple infections represented only 3.6% of infections (n = 5). HPV16 and HPV18 were involved in three out of five multiple infections. Six cases (4.3%) were HPV positive but the types could not be determined.

Comparisons between sub-Saharan African countries and with other regions

Analyses were restricted to ICC cases classified as SCC. No statistically significant difference was found within SSA countries in the RC of HPV16, HPV18 and HPV45 although there was a pattern: the RC of HPV18 was 2- to 3-fold higher in Uganda and Mozambique than in Mali and Senegal (Table 3). Pooled analyses showed that, in SSA, women infected with HPV16, HPV18 and HPV45 as single infections were younger than women infected with other HPV types (51.0 *vs.* 54.5 years old, P = 0.003) (data not shown). They were also younger when pooling single and multiple infections together (51.0 years *vs.* 55.3 years, P = 0.003).

Table 1 HPV DNA detection in invasive cervical carcinoma in Mali and Senegal

Variables	ICC cases tested for HPV		HPV positive cases		
	N	%	*N*	Crude prevalence % (95% CI)	Adjusted prevalence % (95% CI)*
Country					
Mali	32	19.5	28	87.5 (71.0–96.5)	88.2 (71.3–95.7)
Senegal	132	80.5	110	83.3 (75.8–89.2)	86.5 (78.4–91.8)
Period of diagnosis					
2006–2008	86	52.4	75	87.2 (78.3–93.4)	89.5 (80.6–94.2)
2009–2010	78	47.6	63	80.8 (70.3–88.8)	83.2 (72.2–90.5)
Age at diagnosis (in years)					
≤39	22	13.4	18	81.8 (59.7–94.8)	82.2 (60.7–93.3)
40–49	50	30.5	39	78.0 (64.0–88.5)	78.9 (65.2–88.3)
50–59	43	26.2	41	95.3 (84.2–99.4)	95.8 (84.4–99.0)
≥60	47	28.7	38	80.9 (66.7–90.8)	82.0 (68.0–90.7)
Missing	2	1.2	2	100	
Histological type					
Squamous cell carcinoma	150	91.5	129	86.0 (79.4–91.1)	88.0 (80.9–92.6)
Adenocarcinoma	5	3.0	3	60.0 (14.6–94.7)	64.1 (18.8–93.2)
Adenosquamous	5	3.0	4	80.0 (28.3–99.5)	82.4 (32.9–97.8)
Other diagnoses†	4	2.5	2	50.0 (6.7–93.2)	51.0 (11.2–89.5)
Total	164	100	138	84.1 (77.6–89.3)	86.8 (79.7–91.7)

ICC, invasive cervical carcinoma; HPV, human papillomavirus; CI, confidence interval.
*Prevalence adjusted for all the variables in the table. Reference categories for the logistic regression are underlined. Two-sided 95% confidence intervals (95% CI) were calculated for each prevalence estimate.
†Other diagnoses include three undifferentiated carcinomas and one neuroendocrine carcinoma.

Comparisons of HPV distribution between SSA and other regions highlighted that HPV16 was significantly less frequent than in the rest of the world (49.4% *vs.* 61.4%; $P < 0.0001$) while HPV18 was significantly more frequent (19.3% *vs.* 9.4%; $P < 0.0001$) providing overall a similar joint contribution of both types in SSA compared with the rest of the world (68.6% *vs.* 70.8%, $P = 0.24$; Table 3). Table 3 also shows that HPV45 is more prevalent in SSA, compared with the rest of the world (10.3% *vs.* 5.6%, $P < 0.0001$).

Correlation with HIV

Figure 2 illustrates the correlation between the RC of HPV in SCC and 2009 HIV prevalence estimates in the African countries included in the study. Reported HIV prevalence rate in adults aged 15–49 years was 11.5% in Mozambique (10.6– 12.2%), 6.5% in Uganda (5.9– 6.9%), 3.6% in Nigeria (3.3– 4%), 1% in Mali (0.8–1.3%) and 0.9% in Senegal (0.7– 1%) (UNAIDS 2009). We found a positive correlation between HIV prevalence and the RC of HPV18 ($r = 0.9$; $n = 5$; $P = 0.037$) and a negative correlation between HIV prevalence and the RC of HPV45 ($r = -0.9$; $n = 5$; $P = 0.037$). The RC of HPV16 was not correlated with HIV prevalence ($P = 1.0$).

Discussion

In our study in Mali and Senegal, 14 high-risk HPV types (13 involved in single infections and 7 in multiple infections) were detected in ICC. HPV16 was the main type in both countries. The second most frequent type was HPV45 and the third one was HPV18. HPV16 and HPV18 both included in the two prophylactic vaccines currently available accounted for over half of all HPV-positive cervical cancers. Therefore, these data suggest that as many cervical cancers could be averted if vaccination was made widely available. HPV45 is not a vaccine target but contributed to 14.5% of single types detected in cervical tumours. Our data as well as those from other studies in Africa highlight the importance of this HPV type in SCC (Bosch *et al.* 1995; Naucler *et al.* 2004; Odida *et al.* 2008; Castellsagué *et al.*

Table 2 HPV type distribution among 138 HPV positive invasive cervical cases from Mali and Senegal

HPV type	*N*	Relative contribution % (95% CI)
HPV type distribution in single infections		
HPV16	58	42.0 (33.6–50.7)
HPV18	18	13.0 (7.9–19.8)
HPV31	4	2.9 (0.8–7.2)
HPV33	6	4.3 (1.6–9.2)
HPV35	6	4.3 (1.6–9.2)
HPV39	4	2.9 (0.8–7.2)
HPV45	20	14.5 (9.0–21.4)
HPV52	1	0.7 (0–3.9)
HPV56	1	0.7 (0–3.9)
HPV58	3	2.2 (0.4–6.2)
HPV59	2	1.4 (0.1–5.1)
HPV66	1	0.7 (0–3.9)
HPV68 or HPV73	3	2.2 (0.4–6.2)
Total number of single infections	127	92.0 (86.1–95.9)
HPV type distribution in multiple infections		
HPV16 and HPV45	1	0.7 (0–3.9)
HPV16 and HPV51	1	0.7 (0–3.9)
HPV18 and HPV56	1	0.7 (0–3.9)
HPV45 and HPV54	1	0.7 (0–3.9)
HPV45 and HPV56 and HPV66	1	0.7 (0–3.9)
Total number of multiple infections	5	3.6 (1.1–8.2)
Undetermined HPV type		
HPV X	6	4.3 (1.6–9.2)
Relative contribution of HPV 16 and HPV 18 and HPV 45 in all infections		
HPV16 and 18	79	57.2 (48.5–65.6)
HPV16 and 18 and 45	101	73.2 (65.7–81.0)

HPV, human papillomavirus; CI, confidence interval.

2008; Keita *et al.* 2009; De Vuyst *et al.* 2009; Adjorlolo-Johnson *et al.* 2010; de Sanjosé *et al.* 2010; WHO/ICO 2010). HPV45 should be considered a candidate for future vaccines, despite the fact that varying degrees of cross-protection against HPV45 have been described for the current bivalent and quadrivalent vaccines (Ault 2007; Einstein *et al.* 2011). Several clinical trials are ongoing to test two variations of a 9-valent (HPV6/11/16/18/31/33/45/52/58) recombinant HPV L1 VLP vaccine (Merck 2012; Roberts *et al.* 2011).

As expected, SCC had the highest HPV positivity rate (88%) while HPV DNA detection in other histological classifications was somewhat lower (Pirog *et al.* 2000; WHO/ICO 2010). Single infections were largely predominant in Mali and Senegal; only five cases (3.6%) harboured concomitant HPV multiple types. This is not the case in Mozambique where 36% of infections were multiple infections, potentially explained by the presence of underlying accompanying cervical dysplasia or subclin-

Table 3 HPV16, HPV18 and HPV45 relative contribution in squamous cell carcinoma in sub-Saharan Africa and worldwide

	HPV16+		HPV18+		HPV45+		HPV16/18+		HPV16/18/45+		Total cases	Total HPV+
	N	%	*N*	%	*N*	%	*N*	%	*N*	%		
Country												
Western Africa												
Mali	13	48.1	2	7.4	4	14.8	15	55.6	19	70.4	53	27
Senegal	47	46.1	13	12.7	15	14.7	60	58.8	75	73.5	210	102
Nigeria	77	51.7	26	17.4	18	12.1	103	69.1	121	81.2	345	149
Eastern Africa												
Uganda	118	53.2	48	21.6	19	8.6	166	74.8	185	83.3	536	222
Mozambique	96	45.5	48	22.7	17	8.1	144	68.2	161	76.3	466	211
P	0.051*		0.09*		0.27*		0.03*		0.14*			
Region												
All five SSA countries	351	49.4	137	19.3	73	10.3	488	68.6	561	78.9	1610	711
Worldwide without SSA†	5209	61.4	800	9.4	477	5.6	6009	70.8	6486	76.4	18981	8487
P	<0.0001		<0.0001		<0.0001		0.24		0.14			

SSA, sub-Saharan Africa; HPV, human papillomavirus.
*The *P*-values indicate the statistical significance of comparisons among all five SSA countries.
†Mozambique, Nigeria and Uganda: data from de Sanjosé *et al.* (2010).

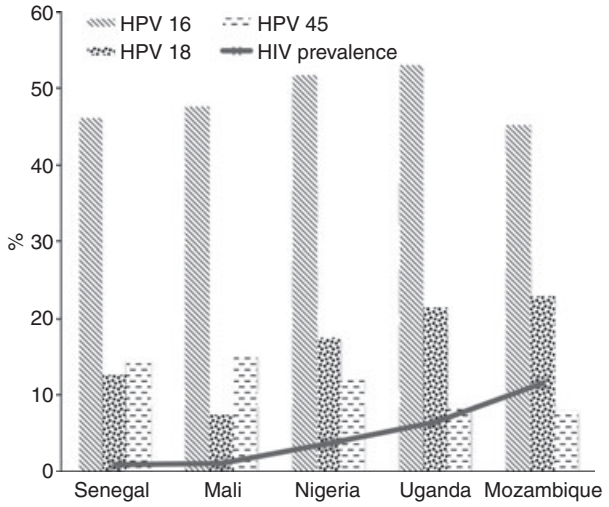

Figure 2 Relative contribution of HPV16, HPV18 and HPV45 in squamous cell carcinoma and HIV prevalence in adults aged 15–49 years. HPV, human papillomavirus.

ical infections in normal mucosa present in the specimen (Castellsagué *et al.* 2008). Existing literature have also reported that multiple HPV infections were more common in HIV-positive *vs*. HIV-negative women with ICC (De Vuyst *et al.* 2012).

The comparison of HPV16, HPV18 and HPV45 distribution between SSA countries and the rest of the world emphasises the importance of HPV18 and HPV45 in Africa. Our study also shows that the RC of HPV18 is higher and HPV45 lower in HIV endemic areas as suggested by the data from Mozambique, Uganda and Nigeria compared with Mali and Senegal which have lower HIV prevalences.

The ICC worldwide study which focused on SCC cases reported that HPV18 was detected in 7% of ICC in Europe and North America, 9% in Central South America and 11% in Asia (de Sanjosé *et al.* 2010). HIV prevalence in these regions is low, and this supports our hypothesis that HIV may influence the distribution of this high-risk type in Eastern Africa. The exception to the rule was Oceania, represented by Australia, where HPV18 was detected in 20% of cases whereas HIV prevalence was reported to be in the lower range with 0.1% (0.1–0.2%) (UNAIDS 2009).

Previous studies in Africa have shown that high-risk HPV types other than HPV16 were more common among HIV-positive women than among HIV-negative women diagnosed with high-grade lesions or ICC (Hawes *et al.* 2003; Banura *et al.* 2011). A study conducted in Kenya and South Africa reported excess HPV18 among HIV-infected women compared to HIV-negative women diagnosed with ICC (De Vuyst *et al.* 2012). Moreover, it has been demonstrated that the immunosuppression induced by HIV infection promotes the severity and progression of pre-neoplasic lesions to cancer (Hawes *et al.* 2003). A possible reason for the higher RC of HPV18 found in countries with higher HIV prevalence could be that the mere fact of having reduced immunity may induce more chances of HPV18 to escape immunity barriers, persist and develop neoplasia and thus increase its relative importance.

The explanation for the inverse correlation between HPV18 and HPV45 in HIV endemic countries is unknown but may be found at the virological level. As these two high-risk HPV types are from the same species (alpha-7 species), we hypothesise that there may be a competitive interaction for viral HPV DNA integration in the host genome in the presence of HIV infection. However, our correlation analyses were made using ecological data for HIV and should be interpreted cautiously.

Invasive cervical carcinoma with HPV16, HPV18 and HPV45 infections were diagnosed at younger age than ICC with other HPV types. The same finding was reported in the worldwide analysis of women diagnosed with ICC (de Sanjosé *et al.* 2010). Our results can be explained by the faster progression from pre-neoplasic lesions to the development of cancer in the presence of these main high-risk HPV types (Trottier *et al.* 2006). Furthermore, HIV may accelerate the rate of progression, thus potentially increasing the number of ICC diagnosed at a younger age in high HIV prevalence areas. The mean age of women diagnosed with ICC was 50.9 years (SD = 12.1) and 51.2 years (SD = 12.3) in Mali and Senegal (low HIV prevalence) whereas it was 44.4 years (SD = 10.6) in Uganda (higher HIV prevalence).

There were a few limitations of our study. Although HPV positivity was expected to be close to 100%, our detection of 86.8% was consistent with the type of retrospective samples retrieved (paraffin-embedded preservation) despite the fact that we used an extremely sensitive assay to detect HPV DNA. We evaluated the quality of our specimens by means of cellular tubulin detection and subsequently excluded the HPV DNA negative and tubulin negative cases from the statistical analyses, but there may exist additional factors that have altered HPV DNA detection such as: storage conditions, number of years since tissue was embedded in paraffin, the type of material used for fixation and substandard processing of samples. Additionally, women with higher socio-economic status whose diagnosis was performed in private institutions were not represented in our study. All of the study cases were selected from the main anatomy and pathology laboratories belonging to public institutions in Senegal and Mali. Despite this fact, our cohort was still

likely to be representative of the target population as the vast majority of women were diagnosed in participant centers.

Finally, it is possible that HPV detection and genotype distribution may be affected by the difference in study periods between the five African countries. For example, cases diagnosed between 1968 and 1992 were used in Uganda whereas only recent cases (1999) were analysed in Mozambique. However, as we excluded cases with no detectable DNA following analysis of the tubulin human gene marker, we minimised low detection rates, which may occur more commonly in old archival specimens.

Our study had several strengths including the use of a common protocol for data collection and testing methods in participating centers and laboratories, allowing for accurate comparisons. Highly sensitive assays were used for HPV detection. Finally, our study reports novel findings on the potential influence of HIV on the RC of the most frequent high-risk HPV genotypes in Western and Eastern Africa.

Conclusion

The importance of HPV16 and HPV18 in ICC and the potential impact of prophylactic vaccines in Western and Eastern Africa have been confirmed by our study. Our results also show the need to consider HPV45 in future polyvalent vaccines as it ranks second in Mali and Senegal and third in the distribution of HPV types in ICC in SSA. Furthermore, the comparisons between SSA countries and other regions of the world suggest that the development of cancer of the cervix in a context of high HIV prevalence needs to be better understood.

Our findings provide the tools for health authorities to understand HPV type distribution and the need to take actions to lower the impact of the disease. It is imperative to accelerate the advent of prophylactic vaccines in SSA. This primary preventive measure, in addition to secondary prevention through early detection of cancer, is an important public health strategy for reducing the burden of the disease. The forthcoming broad-spectrum prophylactic vaccine targeting nine HPV types, if proved efficacious for the additional types, will certainly have a high impact in SSA countries. We recommend a phase III trial in African settings and encourage a rapid translation of results.

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